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## STANDARDIZATION OF SEED BASED GENOTYPING SYSTEM FOR GENETIC PURITY TESTING IN MAIZE

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### ABSTRACT

DNA based characterization of maize genotypes has become the easiest and fastest approach to identify genetic purity as compared to phenotyping. High-quality DNA is a prerequisite for molecular biology experiments and thus DNA extraction is one of the most important steps for several experiments. The conventional DNA source for genotyping is the leaf which requires atleast 2 weeks waiting period from seed planting to leaves sampling. Collection of leaves from the field and labeling are the steps which require time in leaf DNA-based genotyping. The objective of this study is to describe a DNA extraction protocol from seed of hybrids and inbred lines of maize and to determine the suitability of extracted DNA for Polymerase Chain Reaction (PCR). In the present study, the protocol was developed for extracting high quality genomic DNA from seeds with minor modifications without the usage of liquid nitrogen. The concentration of DNA extracted from seeds ranged from 400 ng/ $\mu$ l to 800ng/ $\mu$ l. The results revealed that DNA extracted from seeds was suitable for SSR-PCR analysis. This seed-based method of genomic DNA extraction takes less than 24 hours from sampling to quantification and genotyping. It also avoids germination and waiting for leaf sampling and saves field space.

**Key words:** Seed, DNA, Genetic purity, PCR, SSR markers.

With the advancement in plant biotechnology, molecular marker technology has been useful in classifying germplasm into heterotic groups through DNA fingerprinting, identifying genes or genomic regions associated with biotic and abiotic resistance or tolerance, and germplasm improvement through marker-aided selection. It is necessary to extract the high-quality DNA with good yield for the above mentioned studies. High quality of DNA is essential for molecular based activities such as Polymerase Chain Reaction (PCR), Southern blotting, genomic library construction to sequencing (Aziz *et al.*, 2020). The quality of DNA should be consistent to allow a proper genetic analysis from several individual plants.

Genetic purity is one of the quality criteria required for successful hybrid seed production and identification of genetically distant parental combinations (Eminur *et al.*, 2015). It is estimated that the yield per hectare will decrease by about 135 kg if hybrid seed purity of maize decrease by 1%. Therefore, testing the

genetic purity of hybrid seed is an essential requirement for its commercial use (Chaudhary *et al.*, 2018). Conventionally, testing of genetic purity is being done based on specific morphological and floral characters in plants grown until maturity through Grow Out Test (GOT). This method is time consuming, restricted to a few characteristics and influenced by environmental factors. SSR markers are useful in the identification of hybrid, assessment of respective parents, and testing genetic purity of hybrids (Mawgood *et al.*, 2006, Sudharani *et al.*, 2014, Daniel *et al.*, 2012, Awaludin *et al.*, 2013).

A simple and rapid DNA extraction method is needed for studies such as genetic purity testing of hybrid seeds. Several methods have been reported for minimizing the DNA extraction steps (Berthomieu and Meyer 1991, Edwards *et al.* 1991, Horne *et al.* 2004), but these steps require grinding of a large amount of plant tissue in liquid nitrogen. Further, leaf DNA-based genotyping requires growing the plants in

the field or greenhouse, collecting leaf tissue from the plants, and tracking back to the desirable plants after genotyping. Seed DNA based genotyping is an important alternative that could reduce cost and increase the efficiency of molecular breeding. It also eliminates the need for plant germination and can also aid in testing seed purity before the growing season. Therefore, the current protocol was standardized for efficient extraction of DNA from the seed which consistently produced pure and high-quality DNA suitable for further molecular analysis.

## MATERIAL AND METHODS

### Seed Material

Four hybrids along with their five parental inbreds were selected for the extraction of DNA from the seed to test the genetic purity. The hybrids along with their parental inbreds are listed in Table 1. The seed material was procured from Maize Research Centre, Agricultural Research Institute, Rajendranagar and the experiment was carried out at Institute of Biotechnology, PJTSAU, Rajendranagar.

**Table 1. List of Maize hybrids along with their parental inbred lines used in the study**

S. No	Name of hybrid	Female parent	Male parent
1.	DHM 117	BML-6	BML-7
2.	DHM 121	BML-45	BML-6
3.	KNMH-131	PFSR-3	BML-7
4.	KNMH- 141	KML-225	BML-7

### Reagents employed for the extraction of genomic DNA

Extraction buffer containing 2% CTAB (w/v), 1.4 M NaCl, 1M Tris-HCl pH 8.0, 0.5 M EDTA pH 8.0

0.3% 2-β -Mercaptoethanol

Chloroform: isoamyl alcohol (24:1)

Ice cold 100% isopropyl alcohol

70% ethanol

1× TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0, autoclaved).

Agarose (molecular grade)

### List of equipment used

Qiagen Tissue Lyser II

3/32-inch 440C Stainless steel balls grinding grade (2.38 mm)

Sigma-aldrich Microcentrifuge 1-15

Nanodrop ND-1000 Spectrophotometer (Thermo fischer Scientific)

Biorad Gel documentation system XR+

### DNA extraction protocol from Maize seeds

- The CTAB extraction buffer was pre heated in water bath at 65°C for 5 minutes. 0.3% of 2-β -mercaptoethanol was added to CTAB extraction buffer immediately before use. Warm buffer provides favorable conditions for all the components and helps in breaking up the different tissues of seeds. To ensure high yield and quality, buffer should not be cold.
- 1000 μl of pre warmed extraction buffer was taken and poured into 2 ml microcentrifuge tube.

➤ Approximately 50-100 mg of seeds were taken and transferred into the microcentrifuge tube and crushed with the help of a QIAGEN tissue lyser (30 strokes per second for 2 minutes).

➤ The sample was incubated in water bath at 65°C for 40 min, with mixing after every 5 min.

➤ An equal volume of Chloroform and Iso amyl alcohol (24:1) was added, mixed by slight inversion and the tubes were centrifuged at 12000 rpm for 10 minutes.

➤ Using a micropipette, the upper aqueous phase was transferred into a new 1.5 ml microcentrifuge tube.

➤ The above step was repeated twice to achieve the clear aqueous phase.

## STANDARDIZATION OF SEED BASED GENOTYPING SYSTEM

- The DNA was precipitated by adding cold isopropanol and inverted gently until the formation of DNA threads.
- The tubes were incubated at -20°C for about 30 minutes. Later they were centrifuged at 12000 rpm for about 10 minutes.
- The supernatant was discarded gently without disturbing the DNA pellet.
- The DNA pellet was washed with 200  $\mu$ l of 70 % ethanol and centrifuged at 4000 rpm for 5 minutes.
- Ethanol was discarded gently and the pellet was air dried at room temperature.
- The DNA pellet was re-suspended in 60  $\mu$ l of 1x TE buffer and stored at -20°C until further use.

### Qualitative and Quantitative analysis of the genomic DNA extracted through the above protocol

Quality of DNA extracted from maize seed was evaluated by electrophoresis separation on a 0.8 % agarose gel stained with ethidium bromide with diluted uncut ladder DNA as standard. Concentration and purity of DNA was measured by monitoring absorbance ratio at A<sub>260</sub>:280 nm and A<sub>260</sub>:230nm by Nanodrop 1000 spectrophotometer using 1  $\mu$ l of each sample.

### Polymerase Chain Reaction and electrophoresis for SSR analysis

The genomic DNA extracted from seeds was amplified through SSR primers for the confirmation of hybrid purity. PCR based amplification was carried out in a (10  $\mu$ l) reaction mixture. The DNA concentration was standardized at 100 ng/ $\mu$ l for PCR. The reaction mixture contained the following components: 2  $\mu$ l of template DNA, 0.5  $\mu$ l of forward and 0.5  $\mu$ l of reverse primers, 4  $\mu$ l of TAKARA premix (PCR buffer, Taq polymerase, MgCl<sub>2</sub> and DNTP's) and 3  $\mu$ l of molecular grade water. Amplification reactions were carried out in a thermal cycler in the following manner: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 45 sec. and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The amplified PCR products were electrophoretically resolved on 3% agarose gel stained with ethidium bromide in 1xTAE buffer with 100 volts for 2 hours. DNA banding patterns

were visualized using BIO-RAD Imaging gel documentation system.

## RESULTS AND DISCUSSION

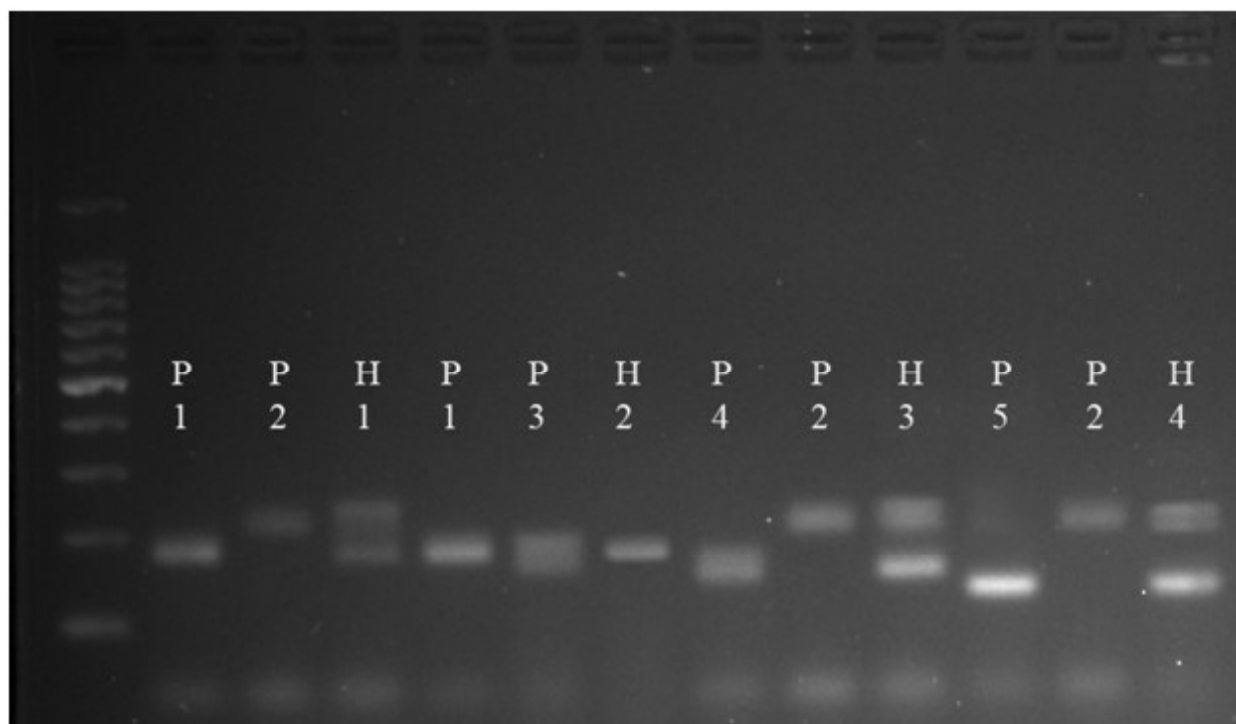
To test the efficacy of the above protocol, the quality and quantity of DNA extracted was examined through agarose gel electrophoresis and Nanodrop 1000 spectrophotometer. The Nanodrop absorbance profile is useful for detection of contaminants such as protein, salts and polysaccharides, which can inhibit and interfere in DNA amplification. The purity of DNA was determined by calculating the ratio of absorbance at 260/280 nm and 260/230 nm and DNA concentration was calculated in ng/ $\mu$ l. Ratio of absorbance at 260/280 nm indicates the presence of proteins and ratio of absorbance at 260/230 nm indicates the presence of phenols and polysaccharides. The results obtained indicated that high quality of DNA free from proteins, phenols and polysaccharides is obtained. The DNA pellet obtained was white and clear without any colouration. The concentration of DNA ranged from 400 ng/ $\mu$ l to 800 ng/ $\mu$ l (Table 2). The ratio of absorbance at 260/280 ranged from 1.6 to 1.8 indicating that the isolated DNA is free from protein contamination. On the other hand, the ratio of absorbance at 260/230 ranged from 1.2-2.0.

To test the suitability of the extracted genomic DNA for PCR- based analysis, it was screened with randomly selected SSR markers. The results revealed that extracted genomic DNA from the seed tissues was successfully amplified by SSR primers which implies that it is of good quality and is suitable for SSR-PCR analysis. SSR primer *bnlg* 1185 confirmed the purity DHM-117, KNMH-131 and KNMH-141 whereas SSR primer *bnlg* 2042 confirmed the purity of DHM-121. (Figure 1 and Figure 2). Genotyping through DNA extracted from seed by PCR analysis in rice and wheat was reported by Kang *et al.*, (1998). They tested for PCR amplification with addition of proteinase K in the PCR buffer. In the present study, high quality DNA free from proteins was obtained without the addition of proteinase K. Similar result was obtained by Adetumbi *et al.*, (2013) who stated that DNA extraction from maize seed is a suitable for the amplification and PCR analysis.

The current protocol developed is both quick and economical and well suitable for routine analysis of a large number of samples by the technicians in

**Table 2. Quality and quantity of DNA extracted from hybrids and inbred lines in maize**

S.No	Hybrid/Inbred line	Concentration (ng/ $\mu$ l)	OD <sub>260/280</sub>	OD <sub>260/230</sub>
1	DHM-117	706.9	1.80	1.85
2	DHM-121	702.7	1.77	1.25
3	KNMH-131	789.6	1.61	1.47
4	KNMH-141	569.0	1.69	1.20
5	BML-6	402.7	1.71	1.80
6	BML-7	514.2	1.80	1.29
7	BML-45	464.5	1.74	1.20
8	PFSR-3	688.7	1.60	1.35
9	KML-225	691.8	1.67	1.53



L- Ladder (100bp) P1 – BML 6 : P2 – BML 7 : P3 – BML 45 : P4 – PFSR 3 : P5 – KML 225  
 H1 – BML 6 × BML 7- (DHM 117) : H2 - BML 6 × BML 45 - (DHM 121)  
 H3 – PFSR 3 × BML 7 - (KNM 131) ; H4 – KML225 × BML 7 - (KNM 141)

**Figure 1. Confirmation of purity of hybrids DHM-117, KNMH-131 and KNMH-141 through seed genomic DNA**

seed testing laboratories. This simple protocol also avoids dependence on ready-to-use DNA extraction kits, supplied by a number of manufacturers which involves high cost.

### CONCLUSION

The protocol formulated in the present study is suitable for extracting high quality DNA from seeds.

The obtained DNA can be easily used for PCR analysis without any need for further purification. By adopting this method, large number of samples can be extracted in 24 hrs, which will be useful for assessment of genetic purity of seed lots during seed multiplication as well as in breeding programmes that require rapid screening of large population. This was also found to be very useful in molecular studies as a

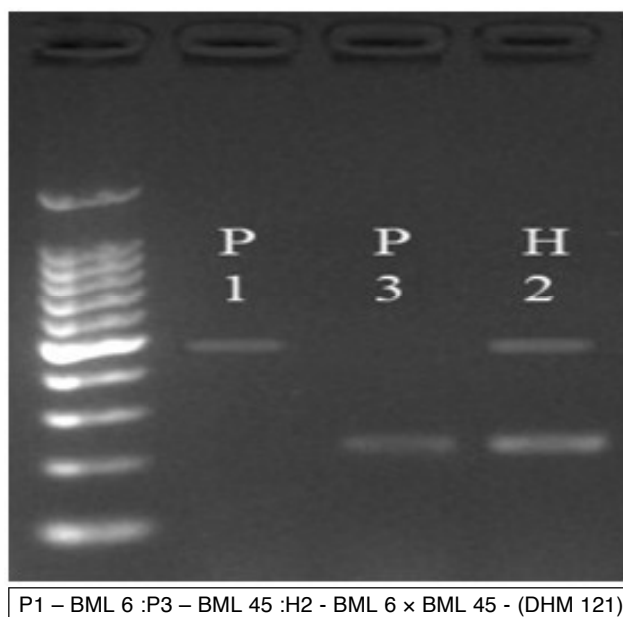


Figure 2. Confirmation of purity of hybrids DHM-121 through seed genomic DNA

suitable alternative to DNA extraction from leaves specially to overcome the problem related to the seed germination when the seeds are old and of low viability.

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## VARIABILITY AMONG DIFFERENT ISOLATES OF *Sclerotium rolfsii* Sacc. ASSOCIATED WITH STEM ROT DISEASE OF GROUNDNUT

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### ABSTRACT

Groundnut (*Arachis hypogea* L.) the king of oilseeds remains as the valuable source of all nutrients. Stem rot caused by *Sclerotium rolfsii* is a destructive disease responsible for serious yield losses in groundnut around the world. Variation in morphological and cultural characteristics of thirty isolates of *Sclerotium rolfsii* were studied based on their growth rate, colony type, growth type, sclerotial pattern, sclerotial type, scleroial color and sclerotial size using potato dextrose agar (PDA). Significant variability with reference to mycelial and sclerotial characters across isolates of *S. rolfsii*, collected from Warangal, Wanaparthy and Nagarkurnool districts of Telanagna was observed. The growth rate ranged from 0.76 to 1.35 mm/hr. All the isolates produced sclerotia on PDA medium. Most of the isolates produced the colonies which were raised at ends ( $n=14$ ) followed by flat type ( $n=12$ ) and raised type ( $n=4$ ). As per mycelial growth type, most of the isolates were found highly profuse in growth ( $n=18$ ) and few were profuse in growth ( $n=12$ ). Likewise, the isolates exhibited considerable variation with respect to morphological characteristics. Wide variation was also found with respect to number of sclerotia per plate (58 to 536), pattern of sclerotia produced in Petri dish (scattered category ( $n=23$ ) and peripheral ( $n=7$ )), colour of sclerotia (brown colour ( $n=13$ ) followed by dark brown ( $n=9$ ) and light brown ( $n=7$ )), size of sclerotia (0.17 mm to 2.34 mm). Principal Component Analysis extracted four main components, growth rate, colony type, growth type and sclerotial pattern, from the population that described the variability in the population most appropriately.

**Key words:** Stem rot, *Sclerotium rolfsii*, Groundnut, Morphological variability, Cultural Variability, Principal Component Analysis.

Groundnut (*Arachis hypogea* L.) is important oilseed crop grown in India, China, Nigeria, Senegal, Sudan, Burma and The United States of America. Peanuts are rich in energy (567 calories per 100 g) and contain health benefiting nutrients, minerals, antioxidants and vitamins that are essential for optimum health. The kernels are a good source of dietary protein, compose fine quality amino acids that are essential for growth and development. They compose sufficient levels of mono-unsaturated fatty acids especially oleic acid. It helps to lower LDL or "bad cholesterol" and increase HDL or "good cholesterol level in the blood.

Groundnut is grown to an extent of 29.59 Mha worldwide with a total production of 48.75 MT (FAOSTAT, 2019). In India, the crop is grown to an extent of 4.8 Mha with a production of 9.2 MT (INDIASTAT, 2019). In Telangana state, it is grown to an extent of 0.13 Mha with a production of 0.30 MT

and productivity of 2364 kg ha<sup>-1</sup> (Directorate of Economics and Statistics, 2019).

Soil borne diseases have been recognized as one of the major limiting factors for groundnut production. *Sclerotium rolfsii* is a soil borne plant pathogen causing root rot, stem rot, collar rot, wilt and foot rot diseases on more than 500 plant species of agricultural and horticultural crops throughout the world (Aycock, 1966). Stem rot has been observed, where moisture and temperature conditions are sufficiently high to allow the growth and survival of *Sclerotium rolfsii*. Groundnut plants were infected by *S. rolfsii* at all growth stage including the germinating stage of the seed causing pre-emergence rot and young plant shown stem rot. The time taken for wilting varied from 8 to 15 days. The younger plants were found more susceptible as the infection was more and rapid (Patil and Rane 1983). The mycelium of *S. rolfsii* survives

best in sandy soils, whereas the sclerotia survive best in moist, aerobic conditions found at the soil surface (Punja, 1985).

There are several reports of *S. rolfsii* which show significant variations in morphological behavior (Sharma *et al.*, 2002). Variability is the property of an organism to change its characters from one generation to the other. This pathogen is host specific and major hindrance to groundnut production in tropics and subtropics and needs information based on the fungal features and variability in culture and pathogenicity. There is need to study and characterize the fungus isolated from different districts of Telangana, where groundnut is grown on a large scale. The information on cultural, morphological and pathological variability among the isolates *S. rolfsii* is limiting.

## MATERIAL AND METHODS

### Isolation and maintenance of the pathogen

Groundnut plants showing typical symptoms of stem rot collected during survey from different districts were used for isolation of *S. rolfsii* separately by tissue segment method (Rangaswami, 1993) using sterile Potato dextrose agar medium. The infected plants showing the presence of white mycelial mat with small round brown sclerotia near the collar region were pulled out and gently tapped to remove the soil and dirt particle. The infected portions of diseased plants collected from different area were cut into small pieces of one cm size using sterilized scalpel. These pieces were surface sterilized with 0.1 per cent sodium hypochlorite for one minute and washed in sterile distilled water thrice and then placed at equidistance in a Petri dish containing solidified Potato dextrose agar medium. These plates were incubated at  $27\pm 1^\circ\text{C}$  in a BOD incubator for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified as *S. rolfsii* based on the morphological characters as described by (Punja, 1985).

### Cultural and morphological variability

Thirty isolates of *S. rolfsii* isolated from Warangal, Wanaparthy and Nagarkurnool districts of Telangana were studied for their cultural and morphological characters like growth rate, colony type, growth type, sclerotial pattern, sclerotial type, scleroial

color and sclerotial size using potato dextrose agar (PDA). The *S. rolfsii* isolates were cultured on PDA medium at  $27\pm 1^\circ\text{C}$ . Petri dishes containing PDA were inoculated in the centre separately with a 6 mm mycelial disc of 5 days old actively growing cultures of *S. rolfsii* isolates. The radial mycelial growth of the fungal colony was determined at 24 and 48 h after incubation. Finally, the radial mycelial growth was expressed as growth rate (mm/h). Observation on pattern of sclerotial production (central, peripheral, or scattered), colour of sclerotial bodies, number of sclerotial bodies produced per plate and sclerotial diameter were recorded at 21 days after incubation. The sclerotial diameter was determined by measuring the size of 30 randomly selected sclerotia from individual isolate using digital vernier caliper (Aerospace Digimatic Vernier Calliper) and mean sclerotial diameter was calculated.

**Statistical analysis:** All the experiments were carried out with three replications under controlled laboratory conditions. Data collected were analyzed by following completely randomized design (CRD). Principal Component Analysis (PCA) for 30 isolates *S. rolfsii* was done by using R software.

## RESULTS AND DISCUSSION

The isolated pathogen was identified as *Sclerotium rolfsii* Sacc. based on mycological characters, the fungal mycelium was first silky white in color later turned to dull white with radial spreading giving fan like appearance. Microscopic examination of the fungal culture revealed the aerial hyaline, thin walled, septate hyphae with profusely branched mycelium showing clamp connections. When fungus attained maturity small mycelial knots were formed, which later turned to mustard seed like sclerotia which were deep brown or brownish black, shiny, hard and spherical to irregular in shape. Similar reports were given by Subramanian (1964), Barnett and Hunter (1972), Mahmood *et al.* (1976), Mirza and Aslam (1993), Kokub *et al.* (2007) and Prasad *et al.* (2010).

### Cultural and morphological variability

Cultural diversity of 30 isolates of *S. rolfsii* was studied with respect to growth rate, colony type, growth type and presence of sclerotia (Table 1). The growth rate of 30 isolates tested exhibited wide range (0.76 to 1.35 mm/hr). Significantly the lowest growth was found in isolate SrKWa (0.76 mm/hr) followed by



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SrBNg (0.8 mm/hr), SrMaW (0.85 mm/hr), SrRW (0.88 mm/hr), SrSWp (0.89 mm/hr), SrKW (0.92 mm/hr), SrZNg (0.92 mm/hr), SrEW (0.93 mm/hr) and SrPNg (0.94 mm/hr) and were on par with each other. The growth rate of SrNW, SrMW, SrDhW, SrLW, SrRW, SrBWa and SrGkNg were on par with each other. Further, significantly highest growth of 1.35 mm/hr was recorded in isolate SrPWp.

All the 30 isolates of *S. rolfsii* under study produced sclerotia on PDA medium. The isolates found diverse with respect to colony type. Most of the isolates produced the colonies, which were raised at ends ( $n=14$ ) followed by flat type ( $n=12$ ) and raised type ( $n=4$ ) (Figure 1). As per the mycelial growth type, most of the isolates were highly profuse in growth ( $n=18$ ) and few were profuse in growth ( $n=12$ ).

Morphological diversity of 30 isolates of *S. rolfsii* was studied with respect to pattern of sclerotial production in Petri dish, color of sclerotia, number of

sclerotia per plate, and size of sclerotia (Table 1). As per the pattern of sclerotia produced in Petri dish, most of the isolates were fell into scattered category ( $n=23$ ) and rest peripheral ( $n=7$ ) (Figure 2). The colour of sclerotia was ranged from brown to dark brown, most of isolates produced brown colour sclerotia ( $n=13$ ) followed by dark brown ( $n=9$ ) and light brown ( $n=7$ ).

The number of sclerotia produced per Petri dish by 30 isolates of *S. rolfsii* ranged from 58 in SrYWa to 536 in SrGkNg. The significantly least number of sclerotia was produced by isolate SrLW (74) followed by SrCWp (76), SrBWa (83), SrNW (84), and SrNW (89) which were at par with each other. Whereas, significantly highest number of sclerotia was produced by isolate SrGkNg (536) followed by SrBNg (492) and SrMW (486) which were at par with each other. There was wide variation in size of sclerotia produced by isolates tested and ranged from 0.17 mm to 2.34 mm. Additionally, most of the isolates ( $n=26$ ) produced the

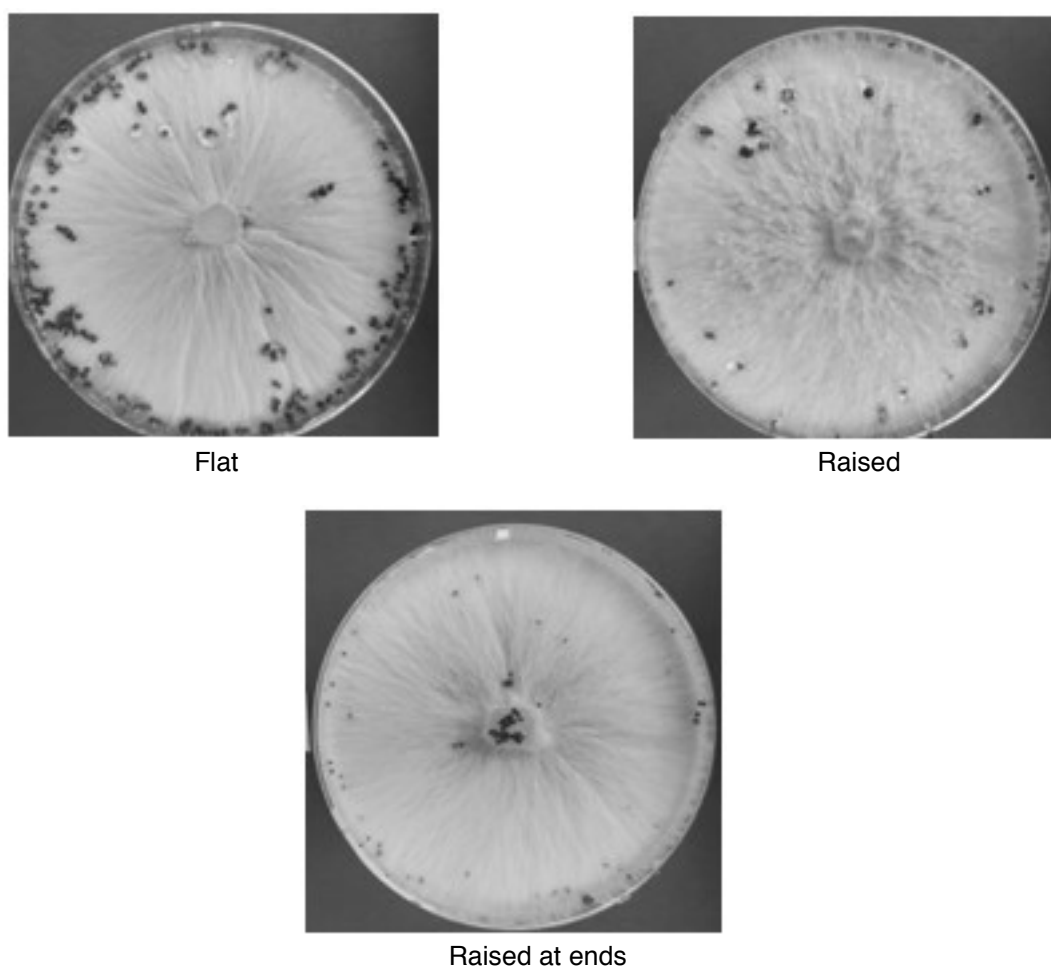


Figure 1. Types of colonies produced by isolates of *Sclerotium rolfsii*

Table 1. Variation among different isolates of *Sclerotium rolfsii* based on cultural and morphological characteristics

S. No	Isolate	Place of collection		Cultural characters			Morphological characters			
		Village	District	Growth rate	Colony type	Growth type (mm/h)	Sclerotial pattern	Sclerotial number	Sclerotial color	Sclerotial size (mm)
1	SrKW	Kadarigudem	Warangal	0.92	Flat	Profuse	scattered	232	brown	1.43
2	SrRW	Ramojikummarigudem	Warangal	0.88	Flat	Profuse	scattered	246	brown	1.54
3	SrKaW	Kammappally	Warangal	1.22	Raised at ends	Highly Profuse	scattered	295	Dark brown	1.98
4	SrNW	Nashkal	Warangal	1.13	Raised at ends	Highly Profuse	scattered	84	Light brown	1.56
5	SrEW	Eilanda	Warangal	0.93	Flat	Profuse	scattered	412	Dark brown	1.65
6	SrMW	Madannapet	Warangal	1.13	Raised	Profuse	peripheral	486	Light brown	1.13
7	SrCW	Chandraypally	Warangal	1.16	Raised at ends	Highly Profuse	scattered	413	brown	1.81
8	SrDW	Dasaripally	Warangal	1.26	Raised at ends	Highly Profuse	scattered	96	Light brown	0.17
9	SrMaW	Malakpally	Warangal	0.85	Flat	Profuse	scattered	412	Dark brown	1.26
10	SrDhW	Dharmapur	Warangal	1.20	Raised at ends	Highly Profuse	scattered	365	brown	1.69
11	SrNW	Nandigama	Warangal	1.27	Flat	Highly Profuse	scattered	89	Light brown	0.42
12	SrMuW	Muchimpla	Warangal	1.21	Flat	Highly Profuse	scattered	106	brown	1.73
13	SrLW	Lenkalapalli	Warangal	1.13	Raised at ends	Highly Profuse	peripheral	74	Dark brown	2.24
14	SrRW	Rangapur	Warangal	1.11	Raised	Profuse	scattered	224	Dark brown	2.01
15	SrPWp	Palem	Wanaparthy	1.35	Raised at ends	Highly Profuse	peripheral	276	brown	1.36
16	SrAWp	Apparaala	Wanaparthy	1.22	Flat	Profuse	peripheral	324	Dark brown	1.73
17	SrPWp	Polikapadu	Wanaparthy	1.06	Raised at ends	Profuse	scattered	194	Light brown	0.63
18	SrSWp	Sankireddypally	Wanaparthy	0.89	Flat	Profuse	scattered	393	brown	1.68
19	SrCWp	Chennur	Wanaparthy	1.24	Raised	Highly Profuse	scattered	76	brown	1.52
20	SrKWa	Kanimetta	Wanaparthy	0.76	Flat	Profuse	peripheral	378	Dark brown	1.59
21	SrYWa	Yedutla	Wanaparthy	1.10	Flat	Highly Profuse	peripheral	58	Light brown	1.12
22	SrBWA	Buddaram	Wanaparthy	1.13	Flat	Profuse	scattered	83	Light brown	2.03
23	SrZNg	Zamisthapur	Nagarkurnool	0.92	Raised at ends	Profuse	scattered	368	Dark brown	1.93
24	SrBNg	Bopally	Nagarkurnool	0.80	Raised	Profuse	scattered	492	brown	1.86
25	SrPNg	Pedduru	Nagarkurnool	0.94	Flat	Profuse	scattered	268	brown	1.82
26	SrGkNg	Gattunallykuduru	Nagarkurnool	1.20	Raised at ends	Highly Profuse	scattered	536	brown	1.22
27	SrGNg	Gattuthumben	Nagarkurnool	1.18	Raised at ends	Highly Profuse	scattered	112	Light brown	0.23
28	SrTNg	Thummanpet	Nagarkurnool	1.06	Flat	Profuse	scattered	392	brown	1.95
29	SrJNg	Jinkunta	Nagarkurnool	1.08	Raised at ends	Profuse	peripheral	412	brown	1.61
30	SrGNg	Godal	Nagarkurnool	1.32	Raised at ends	Highly Profuse	scattered	343	Dark brown	2.34
CD (0.01)				0.07	-	-		34.50	-	0.14
S.E.m.±				0.02	-	-		12.19	-	0.05
CV (%)				4.42	-	-		7.69	-	6.06

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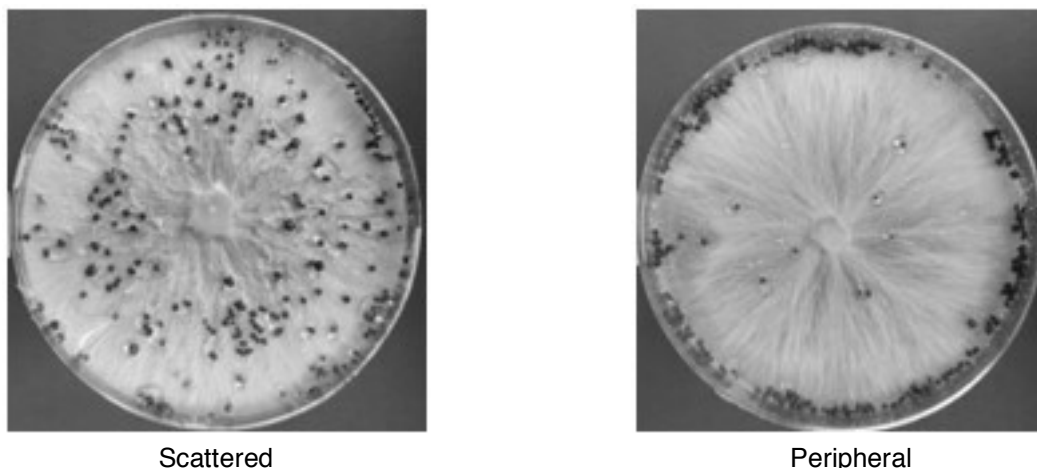


Figure 2. Patterns of sclerotial production by isolates of *Sclerotium rolfsii*

sclerotia of more than one mm in size and few ( $n=4$ ) produced sclerotia of less than one mm in size.

### Principal component analysis

All the 30 isolates were subjected to Principal Component Analysis (PCA) on the basis of different components (growth rate, colony type, growth type, sclerotial pattern, sclerotial number, sclerotial color and sclerotial size) describing the morphological variability in *S. rolfsii* population. PCA extracted three main components from the population. Component 1 described for highest (1.23) Eigen value with variance of 32.86% while the lowest (0.06) Eigen value was observed in component 7 with variance of 1.70%. Different components *viz.*, growth rate, colony type, growth type and sclerotial pattern (component 1, 2, 3 and 4 respectively) accounted for Eigen values  $> 0.5$  whereas, remaining three components accounted for Eigen values  $< 0.5$  (Table 2). Scree plot (Figure 3) graphs the Eigen value against the components described above. Flat line from the fourth component onwards suggests that each successive component

is accounting for smaller and smaller amounts of the total variance.

The results are in line with Sharma *et al.* (2002) who reported wide variability among 26 isolates of *S. rolfsii* collected from different localities in India with respect to colony morphology (fluffy/compact), mycelial growth rate and sclerotial formation (80-500 sclerotia/plate). Similarly, Jyothi (2006) observed the wide variation among the isolates of *S. rolfsii* collected from different crops with respect to growth rate (52.00 to 89.83 mm at 72 hours of incubation). Likewise, Hussain *et al.* (2010) classified the *S. rolfsii* isolates in to very fast, intermediate and slow growing based on their growth rate. Similar type of studies on cultural variability of *S. rolfsii* in different crops such as cowpea, tomato, colocasia, jasmine and groundnut was reported by many researchers (Okereke and Wokocho, 2007; Tortoe and Clerk, 2012; Latha and Rajeswari, 2019; Palaiah and Adiver, 2004). In addition, Chandra Sekhar *et al.* (2017) found variation in colony morphology, colony color, appearance, mycelial growth rate, mycelial growth type, site of production of sclerotia,

Table 2. PCA of different morphological variables of *Sclerotium rolfsii* population

Component	Eigen values	Variance (%)	Cumulative (%)
Growth rate (mm/h)	1.23	32.86	32.86
Colony type	0.78	20.87	53.74
Growth type	0.59	15.69	69.44
Sclerotial pattern	0.50	13.43	82.87
Sclerotial number	0.42	11.21	94.08
Sclerotial color	0.15	4.21	98.30
Sclerotial size (mm)	0.06	1.70	100.00

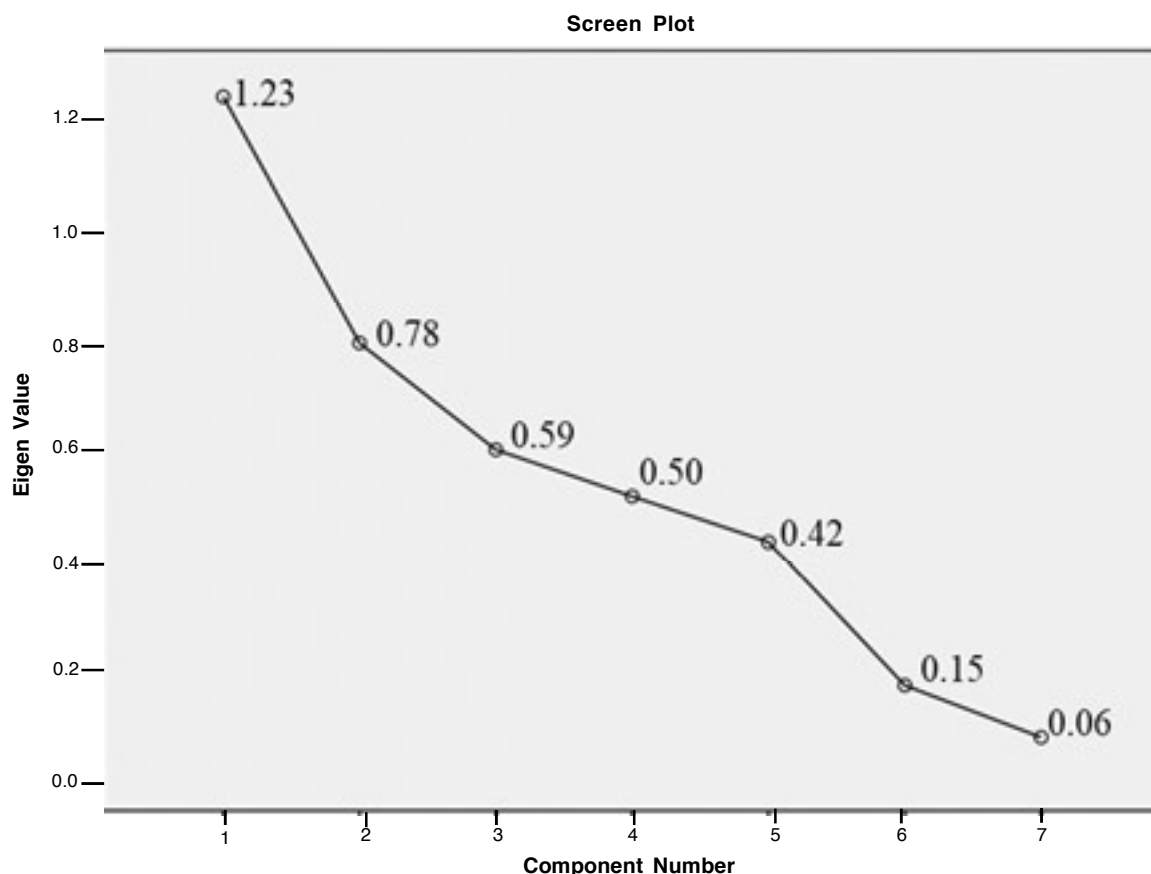


Figure 3. Screen plot showing the Eigen value against the component numbers

number of sclerotia per plate, color of sclerotium and size of sclerotia from the isolates collected from Chittoor district of Andhra Pradesh. Further, Paparu *et al.* (2020) obtained 348 *S. rolfsii* isolates from bean fields in seven agroecological zones of Uganda and found that mycelial growth rate and the number of sclerotia produced on artificial media varied among agroecological zones but not within a zone. The variability among isolates observed in the present study could be attributed to physiometabolic differences among isolates arising from different crop production systems and also some biochemical variability to adapt to their ecological and environmental conditions. Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al.*, 1995; Okabe *et al.*, 1998). PCA in the present study extracted four main components, growth rate, colony type, growth type and sclerotial pattern that can describe the variability in *S. rolfsii* on morphological basis. The present studies revealed variations among isolates of *S. rolfsii*, the causal organism of stem rot of groundnut. However extensive studies are needed with more number of isolates from diversified agro ecological

situations to generate information on pathogenic variation in *S. rolfsii* of groundnut.

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## MULTIVARIATE ANALYSIS OF GENETICALLY DIVERSE GERMPLASM LINES FOR YIELD, GRAIN QUALITY AND PROTEIN CONTENT IN RICE (*Oryza sativa* L.)

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### ABSTRACT

A diverse set of 147 rice genotypes was evaluated for yield, grain protein content (GPC) and grain quality traits. The main objective was to understand the relationship of GPC with yield and important quality traits. Interestingly, GPC had a significant positive correlation with HRR (0.24), indicating that it is possible to identify lines with high GPC and HRR. Further, GPC exhibited significant negative correlation with GC (-0.33) and kernel length (-0.20) and a negative correlation with single plant yield (SPY; -0.12). Head rice recovery % (HRR), another important trait kernel breadth (KB; -0.39) and amylose content (AC; -0.22). Regression analysis showed that GPC in rice grain is mostly influenced by gel consistency (GC), AC, HRR and hulling %. The principal component analysis (PCA) showed that four 'Principal Components' (PCs) contributed to maximum variability (~72.0%) and hierarchical clustering grouped the genotypes into 10 divergent clusters. The genetic diversity among the 147 genotypes evaluated was mostly contributed by Length to Breadth ratio (L:B), kernel length (KL), GC and KB. Clustering indicated that there was high degree of heterogeneity in clusters III to X and this could be exploited in identifying desirable recombinants through hybridization. The results have indicated that through proper evaluation and well planned breeding programs, it is possible to develop high yielding nutritionally enriched rice cultivars that could aid in addressing the both food and nutritional security.

**Key words:** Rice, grain protein content, single plant yield, genetic divergence, head rice recovery

Rice has been a staple food for thousands of years, especially for Asians and Africans. Globally, rice (*Oryza sativa* L.) feeds more than 3.5 billion people worldwide (Fiaz *et al.*, 2019). Rice grains are a source of carbohydrate, protein, fat, dietary fiber, vitamins, and minerals (Pradhan *et al.*, 2019). Rice grain quality has four characteristics, i.e. eating quality, appearance quality, milling quality, and nutritional quality. Rice grain quality parameters like appearance, head rice recovery, cooking/eating, and nutritional traits (ex. grain protein, Fe and Zn) determines the market value (Wu *et al.*, 2020).

Grain protein content (GPC) is a key factor that determines nutritional quality, and is also associated with cooking and eating qualities. The grain protein content (GPC) in rice grain is about 6 - 8% in the commonly consumed milled rice. Among the major cereals like rice, wheat and maize, rice is generally considered as having lower GPC, but rice has the highest net protein utilization rate among the cereal grains (Peng *et al.*, 2014). Also, the amino acid composition in rice grain is relatively balanced. Thus, GPC is an attractive target for enhancing nutritional quality of rice grain.

Apparent amylose content (AAC) measured as the percentage of amylose in total starch is another key determinant of rice cooking properties (Kinoshita *et al.*, 2017); AAC is a complex trait in rice. The presence of many important compounds in the grains of rice has popularized it as golden cereal or to some people as the queen of cereals. As rice is the major source of calories in several countries, developing rice varieties with desirable grain and nutritional properties can help in addressing protein-energy malnutrition affecting about one-third of the world population especially infants and young children. Despite its importance little attention has been given to grain protein content along with yield part of reason could be due lack of high grain protein content varieties in rice. Therefore, identification of sources of high protein is prerequisite for development of high protein varieties. Diversity based on quality characters usually varies with environments and evaluation of these traits requires growing the plants to full maturity prior to identification.

Correlation and regression analyses are useful for assessment of traits under study and their

association, these helps in designing selection strategies for the simultaneous improvement of a combination of traits. At the same time, an assessment of genetic diversity in a set of genotypes under study helps in the development of superior recombinants. While Mahalanobis'  $D^2$ , a powerful tool, quantifies the genetic divergence in germplasm, principal component analysis (PCA) helps in the identification of a set of genotypes capturing maximum genetic diversity of the collection (Gireesh *et al.*, 2017).

Estimation of genetic diversity is a basic prerequisite for successful utilization of desirable traits through breeding. The objectives of this study were to assess the magnitude of variability for yield, grain protein content and quality parameters and thereby identify lines to be used as donors in breeding program.

## MATERIAL AND METHODS

The experiment material comprised of 147 germplasm lines including both *indica* and *japonica* accessions. The experiments were conducted at Ramachandrapuram Farm, ICAR-IIRR, during Kharif, 2019 and 2020 in simple lattice design. All standard recommended management practices were followed. Data was collected on 10 traits related to grain quality and protein content after harvesting at crop maturity. The traits included hulling %, milling %, head rice recovery % (HRR), kernel length (mm; KL), kernel breadth (mm; KB), length to breadth ratio (L:B), amylose content (AC), gel consistency (mm; GC), single plant yield (g/plant; SPY) and grain protein content (%; GPC). The grain quality analysis was carried out at the grain quality lab at ICAR-Indian Institute of Rice Research, Hyderabad as per Aravind *et al.*, 2021. The GPC of all the 147 entries were determined by the standard micro-Kjeldahl method (Yoshida *et al.*, 1976). The grain protein content was calculated by multiplying percent nitrogen content by 5.95.

### Statistical analysis

The correlation and regression analyses were carried out using SAS 9.3 software. Multivariate analysis (Diversity analysis and Principal Component Analysis; PCA) were carried out using 'Biotoools' (Silva, 2017), 'FactoMineR' (Kassambara and

Mundt, 2020) and 'Factoextra' (Husson *et al.*, 2020) R packages.

## RESULTS AND DISCUSSION

### Correlation Coefficients

Correlation studies will be useful in understanding the association between traits (Ishwarya Lakshmi *et al.*, 2019) i.e, here between GPC and quality traits, enabling plant breeders to select accessions possessing desirable traits that are related to GPC. The correlations of different traits are presented in (Figure 1). GPC has significant positive correlation with HRR (0.24) and significant negative correlation with GC (-0.33) and kernel length (-0.20), similar results were observed by Soharu and Pandey (2018) and Khatun *et al.*, (2003). GPC showed a negative correlation with yield (-0.12), KB (-0.15) and AC (-0.08), similar results were reported by Khatun *et al.*, (2003). GC has highly significant positive correlation with kernel length (0.39) and LB ratio (0.32) and a highly significant negative correlation with amylose content (-0.44), Khatun *et al.*, (2003) and Naik *et al.*, (2021) reported similar findings in their experiments.

Another important quality trait, AC exhibited significant negative correlation with HRR (-0.22) and LB ratio (-0.22), while it showed highly significant positive correlation with kernel breadth (0.28), Naik *et al.*, (2021) observed similar results. HRR, an important trait that impacts the market value of a variety exhibited a significant positive correlation with milling % (0.36), and significant negative correlation with KB (-0.39) and AC (-0.22), Suresh *et al.*, (2016) and Naik *et al.*, (2021) reported similar findings previously. Interestingly, HRR showed a significant positive correlation with L:B (0.19) and GPC (0.24). But, Khatun *et al.*, (2003) and Naik *et al.*, (2021) reported a negative relation between HRR and L:B. L:B has significant positive correlation with kernel length (0.79), and highly significant negative correlation with kernel breadth (-0.65) and hulling % (-0.23). Suresh *et al.*, (2016) have observed a positive correlation of HRR with LB and negative correlation with KB, and KL. SPY has significantly positive correlation with hulling % (0.20), KL (0.23), LB ratio (0.26), GC (0.20). Further, SPY has shown a positive correlation with milling %, HRR, and AC, Naik *et al.*, (2021) has reported similar findings.

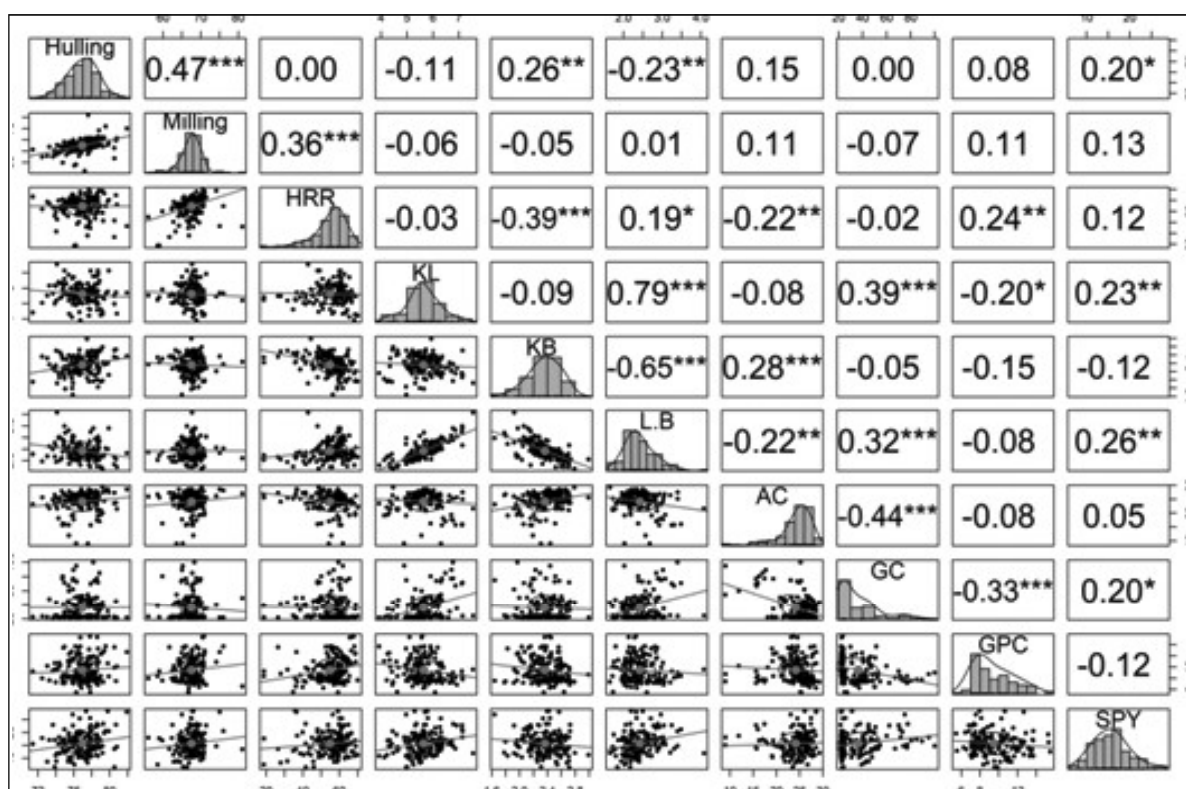


Figure 1. Correlation coefficient between yield, protein content, and grain quality traits

**Stepwise regression analysis of GPC over other traits**

The GPC was influenced by GC (11.12%), AC (6.31%), HRR (3.10%) and Hulling% (1.38%), these four traits together explained 21.91% variability in the GPC (Table 1). Regression equation for GPC is expressed as:

$$GPC = 1.43 - 0.054GC - 0.152AC + 0.041HRR + 0.148Hulling\%$$

With an increase in every one unit of GC, there is a decrease of 0.054 unit of GPC; with an increase in 1 unit of AC, there is a decrease of 0.152 unit of GPC; further for every one unit increase in HRR and hulling%, there is an increase of 0.041 and 0.148 unit of GPC, respectively.

**Principle Component Analysis (PCA)**

The principal component analysis was carried out for the ten different grain characteristics and single plant yield. The PCA was carried out using ‘FactoMineR’ (Kassambara and Mundt, 2020) and ‘Factoextra’ (Husson *et al.*, 2020) R packages. In the PCA, a total of 10 principal components (PCs) were extracted and it revealed four most informative PCs with Eigen values of 2.627, 1.828, 1.596 and 1.149 which accounted for 72.0% of the cumulative variance. The PC’s having Eigen values of more than 1 are considered as most informative PC’s. The first principal component (Dim1) had the highest eigen value of 2.627, explained 26.3% of statistical variance, while second, third, and fourth principal components had eigen values of 1.828, 1.596 and 1.149 explaining 18.3%, 16.0%

**Table 1. Stepwise regression analyses of GPC and other traits in 147 rice genotypes**

Variable	Estimates	SE	F Value	P	Partial R <sup>2</sup>	Model R <sup>2</sup>
Intercept	1.43420	7.18781	9.96	0.8421		0.2191
GC	-0.05392	0.01025	18.15	<.0001	0.1112	
AC	-0.15231	0.05276	11.00	0.0045	0.0631	
HRR	0.04090	0.01773	5.57	0.0225	0.0310	
Hulling	0.14836	0.09361	2.51	0.1152	0.0138	



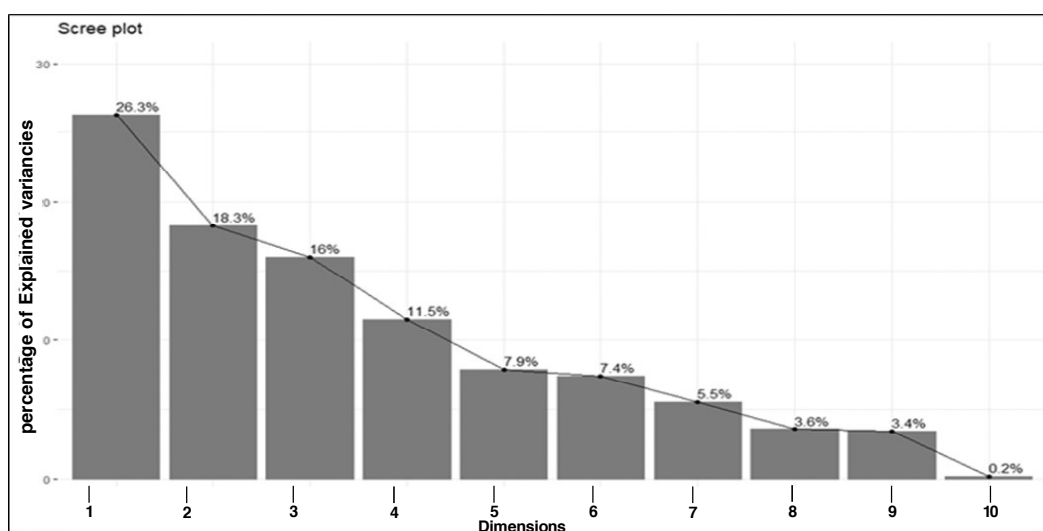
MULTIVARIATE ANALYSIS OF GENETICALLY DIVERSE GERmplasm LINES

and 11.5% of statistical variance respectively (Table 1 and Figure 2a).

of the population under evaluation. Diverse range was detected for cluster means of all the ten traits showing a significant contribution of all the traits in genotypic

**Table 1. Percentage Variation explained by principal components**

Components	Eigen Value	Percentage Variation	Cumulative PercentageVariation
PC1	2.627	26.27	26.27
PC2	1.828	18.28	44.56
PC3	1.596	15.96	60.52
PC4	1.149	11.49	72.01
PC5	0.790	7.90	79.92
PC6	0.743	7.43	87.35
PC7	0.553	5.53	92.89
PC8	0.355	3.55	96.44
PC9	0.338	3.38	99.82
PC10	0.017	0.17	100.00



**Figure 2a.** Screen plot of principal components

As per the PCA variables plot (Figure 2b) and PCA biplots (Figure 2c) four traits viz., L:B, kernel length, kernel breadth, and gel consistency contributed maximum variations in PC1, while three traits viz., milling %, HRR and GPC contributed maximum variations in PC2 (Table 2). Thirty-six genotypes in PC1 has PC scores ranging from 1.03 (G11) to 4.83 (G121), while thirty-three genotypes in PC2 ranging from 1.00 (G3) to 3.42 (G43). Four genotypes (G127, G121, G130, G144) in PC1 and three genotypes (G23, G45, G43) in PC2 contributed to the maximum genetic diversity (Figure 2c).

**D<sup>2</sup>Analysis (Genetic Diversity and Clustering)**

Mahalanobis D<sup>2</sup> statistics is an effective statistical tool that clearly identifies the diverse nature

**Table 2. Contribution of characters to the total divergence in rice genotypes**

Traits	Latent Vectors	
	PCI	PCII
L:B	31.755	0.2
Kernel length	19.82	3.68
Kernel breadth	15.01	10.22
Gel consistency	12.43	0.2
Milling%	0.16	24.59
HRR	3.19	29.5
GPC	1.1	18.78
% Variance	83.465	87.17

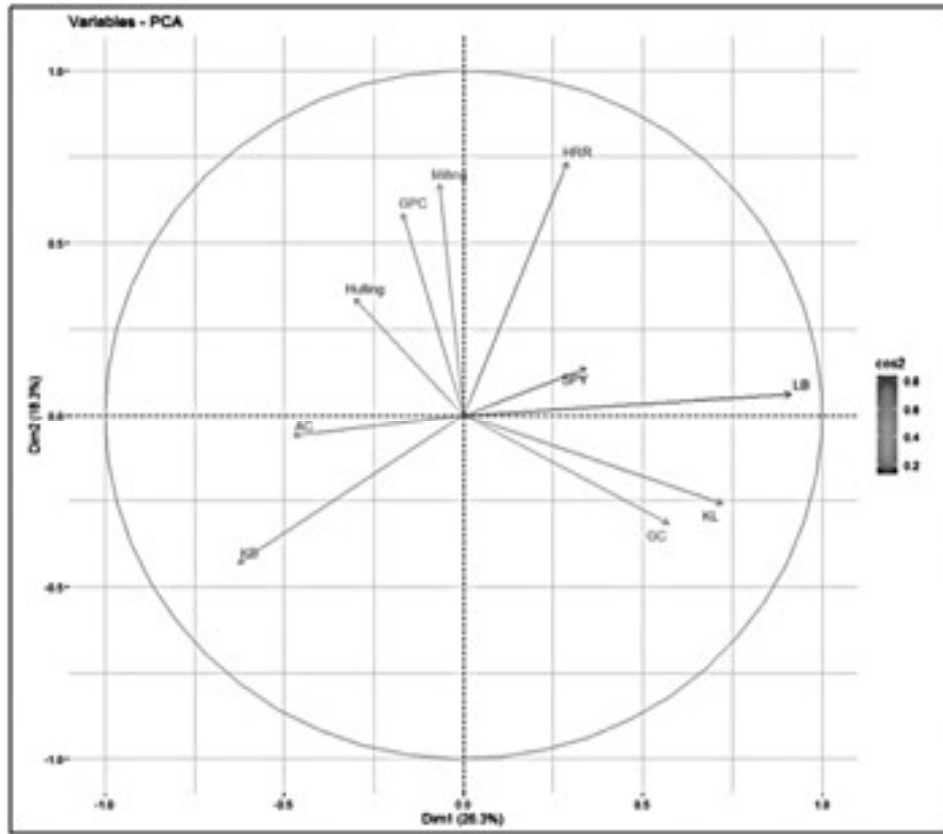


Figure 2b. PCA - loading plot

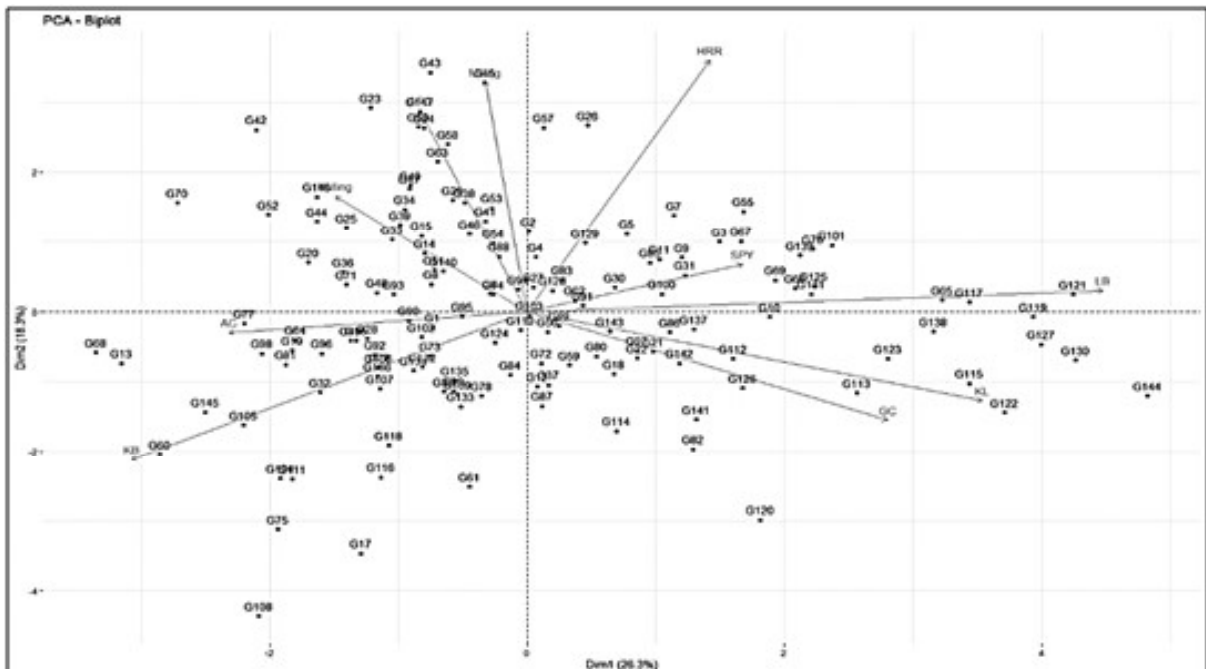


Figure 2c. PCA - Biplots explaining the contribution of 10 quality traits to the total variation in the 147 rice genotypes



traits (Deepika *et al.*, 2021). Further, divergent genotypes would produce a broad variability enabling further selection and improvement which could lead to high levels of heterosis or production of desirable transgressive segregants.

Mean performance of different clusters for the characters studied (Table 3) reflected that all the high GPC genotypes were grouped into cluster X (12.66) followed by II (9.78), IX (9.57) and I (9.50), whereas cluster V (6.53) included low GPC genotypes indicating maximum contribution of these character towards the divergence between cluster X and V. All the high yielding genotypes were grouped into cluster X (27.94) followed by cluster IV (26.94), whereas cluster IX (11.94) was included with low yielding genotypes. Whereas, cluster II (27.24) was found divergent from the cluster

VI (61.91) mainly due to HRR indicating maximum contribution of this character towards the divergence. In this context, Jagadev *et al.*, (1991) reported that the characters contributing maximum towards the divergence should be given greater emphasis for deciding the type of cluster for the purpose of further selection and the choice of parents for hybridization.

The intra cluster degree of diversity ranged from 0 (III-X) to 17.78 in cluster II indicating that the genotypes in cluster II were more heterogeneous than other clusters which were comparatively more closely related (Table 4). The highest inter cluster degree of diversity (125.17) was observed between pairs of clusters III and V followed by III and VII (105.35) and V and IX (99.75) which reflects wider diversity among these clusters. Crosses comprising genotypes from

**Table 3. Cluster means for quality traits of rice genotypes**

Traits	Cluster Means									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Hulling%	76.91	77.50	76.62	75.88	77.61	76.26	77.56	78.17	74.13	81.91
Milling%	67.69	58.84	68.18	65.01	81.07	65.25	63.63	68.34	67.75	75.05
HRR%	55.83	27.24	56.96	56.44	36.14	61.91	54.18	59.45	42.85	36.55
KL	5.63	5.42	4.44	4.97	5.21	4.89	6.31	6.77	7.55	5.69
KB	2.37	2.47	2.72	1.64	2.44	2.44	2.31	1.97	1.92	2.57
LB	2.41	2.19	2.02	2.97	2.10	2.02	2.68	3.40	4.08	2.33
AC	24.26	26.40	26.36	23.08	26.96	9.03	9.01	25.20	21.63	28.57
GC	35.39	26.75	33.00	75.00	50.00	95.00	54.50	100.00	24.50	22.00
GPC	9.50	9.78	6.82	7.54	6.53	8.72	7.41	8.97	9.57	12.66
SPY	15.63	14.20	20.46	26.94	13.45	18.15	15.09	14.81	11.94	27.94

**Table 4. Average intra and inter cluster distance ( $D^2$ ) for rice genotypes**

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	15.85	32.01	63.38	39.29	58.63	37.11	37.12	38.26	55.99	38.68
II		17.78	87.47	48.33	93.86	58.95	46.04	55.11	69.33	48.41
III			0.00	63.48	125.17	68.11	105.35	84.92	63.59	75.44
IV				0.00	84.99	43.67	73.21	32.39	78.73	58.58
V					0.00	84.66	96.00	81.02	99.75	51.64
VI						0.00	29.29	57.44	95.23	69.93
VII							0.00	78.84	74.48	69.79
VIII								0.00	68.93	68.57
IX									0.00	64.73
X										0.00

these clusters would yield desirable recombinants i.e., greater the distance between two clusters, greater would be the genetic diversity among the genotypes (Deepika *et al.*, 2021). Minimum distance was noticed between VI and VII (29.29) followed by I and II (32.01). The inter-cluster distances in all the cases were greater than the intra-cluster distances suggesting wider diversity among the genotypes of the distant groups (Table 4). The similar results regarding inter and intra cluster distances have been described by Islam *et al.*, (2003) in rain fed low land rice.

## CONCLUSION

Interestingly, the results indicate that it is possible to identify lines with high GPC coupled with moderately high grain yield, desirable HRR and probably with also desirable grain type. Step-wise regression analysis indicated that four traits (GC, AC, HRR and hulling %) influence the GPC in rice grains. Also, four traits (L:B, KL, KB and GC) were found to be important for the genetic divergence of the genotypes evaluated (explained ~80% variance). Further, a high degree of heterogeneity was observed during clustering of the genotypes indicating that genotypes in the single genotype clusters could be directly utilized as parents in future hybridization programmes for desirable traits.

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## ASSOCIATION PATTERN AND REGRESSION ANALYSIS FOR YIELD AND ITS ATTRIBUTING TRAITS IN SAFFLOWER (*Carthamus tinctorius* L.)

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### ABSTRACT

The present study was conducted to determine the interrelation among yield and its related traits in safflower using a RIL population derived from the cross between CO-1 and EC-523368-2. The RILs were phenotyped for seven traits, days to 50% flowering, days to maturity, plant height, number of primary capitula/plant, number of seeds/primary capitulum, 100 seed weight and seed yield. Trait mean in the population was observed as 82.08 days for days to 50% flowering, 112.15 days for days to maturity, 75.62 cm for plant height, 6.71 primary capitula/ plant, 28.96 seeds/ primary capitulum, 3.29 g for 100 seed weight. Mean of seed yield was observed as 9.44 ranging from 1.99 to 34.38 g. Correlation analysis revealed the strong positive correlation of seed yield with number of seeds/ primary capitulum, number of primary capitula/ plant, plant height and 100 seed weight. The stepwise regression analysis explained 47% of variation for seed yield (coefficient of determination  $R^2=0.47$ ) and adj.  $R^2$  as 0.45 and developed a best fit linear regression equation as,  $SY = -9.55 + 0.06PH + 0.77NPCP + 0.21NSP + 1.06HSW$ . Based on Mallows' Cp (low- 4.18),  $R^2$  (high- 87.45) and adj.  $R^2$  (high-87.01), the best fit regression model from all possible models was PH + NPCP + NSP + HSW. Selection for these traits may help to improve the seed yield potential in safflower.

**Keywords:** Correlation, stepwise regression analysis, yield traits, safflower, RILs

Safflower (*Carthamus tinctorius* L.),  $2n=24$  belongs to family Asteraceae and is one of the oldest oilseed crops domesticated in India and around the world. It is vernacularly known as 'kusuma' in telugu. Earlier, it has been grown for preparation of dyes from its petals for dyeing (textile and culinary) industries and for preparing medicinal beverages; however, with the advent of artificial dyes, it is grown as oil seed and bird feed (Menegaes and Nunes, 2020). Safflower oil is rich in unsaturated fatty acid, linoleic acid (75%) which is essential for human nutrition (Fernandez-Martinez *et al.*, 1993). It is mainly grown in arid and semi-arid regions with residual moisture as it is having a deep tap-root system. It is primarily a self-pollinated crop and is herbaceous with primary and secondary branches, which terminally bear capitulum (flower head), consisting of 20 to 250 florets (Golkar and Karimi, 2019). The florets in capitulum have different petal range of 5 – 40% cross pollination (Kumari and Pandey, 2005). Presence of different petal colours like yellow, red, orange and white is due to the presence of two natural pigments called carthamin and carthamidin

(Golkar., 2018). The seed is called achene and each flower head commonly contains 15-50 seeds; however, the number can exceed 100 seeds. The crop is majorly spiny and there are commercial varieties which are non-spiny in nature (Singh and Nimbkar, 2018). The crop is under minor cultivation in about 0.52 lakh ha area with 0.44 lakh ton production and 843 kg/ha productivity in India and in Telangana with 0.22 lakh ha area (INDIASTAT, 2021). Productivity of safflower in India is lower than other countries (Jegadeeswaran *et al.*, 2021). Yield being a complex character is influenced by many genetic and non-genetic parameters (Dhage *et al.*, 2020). To improve the yield, the interrelations between the traits help in identification of the selection indices which indirectly put forth towards the yield (Pavithra *et al.*, 2016). Similarly, regression analysis establishes link between two or more variables. It attempts to explain the influence of independent variable on the dependent /response variable, also describes the type and intensity of the relation between the variables (Zvizdojevic and Vukotic, 2015). The present study was carried out to interpret

the relationship and regression between yield and its traits in a RIL population to improve the selection indices in safflower in order to facilitate yield improvement by simple selection programmes.

## MATERIAL AND METHODS

The present study was conducted using a RIL population developed from the cross, CO-1 x EC-523368-2 in *Rabi*, 2020-21. The parents, CO-1 is a popular variety released from Tamilnadu Agriculture University (TNAU) and EC-523368-2 is a selected line from exotic accession received from United States Department of Agriculture (USDA). The experiment was laid out in Augmented Block Design with 145 RILs ( $F_{10}$ ) and the parents, CO-1 and EC-523368-2 were used as checks at the research farm of ICAR-IIOR located at ICRISAT campus. Each RIL line was raised in a single row of 3m length which consisted of 15 plants per row with 45 cm x 20 cm spacing and the observations were recorded on randomly selected five plants in a row. Data were collected for seven traits namely, days to 50% flowering, days to maturity, plant height (cm), number of primary capitula/ plant, number of seeds/ primary capitulum, 100 seed weight (g) and seed yield/ plant (g). Correlation analysis was done to evaluate the degree of association between the yield and its related characters. Statistical analysis of correlation was done using R software (<https://www.R-project.org/>) (R core team 2021).

Correlation coefficients were calculated to determine the degree of association of yield components with seed yield and among themselves. The mean values were used to calculate the coefficients at phenotypic level using the formulae given by Weber and Moorthy (1952).

Phenotypic coefficient of correlation ( $r_p$ ) =

$$r(x_i, x_j)_p = \frac{\text{Cov.}(x_i, x_j)_p}{\sqrt{v(x_i)_p \cdot v(x_j)_p}}$$

Where,

$r(x_i, x_j)_p$  - phenotypic correlation between  $i^{\text{th}}$  and  $j^{\text{th}}$  characters;  $\text{Cov.}(x_i, x_j)_p$  - phenotypic covariance between  $i^{\text{th}}$  and  $j^{\text{th}}$  characters;  $v(x_i)_p$  - phenotypic variance of  $i^{\text{th}}$  character;  $v(x_j)_p$  - phenotypic variance of  $j^{\text{th}}$  character. But in R software, Pearson correlation coefficient was used. The formula used by the software after giving the code in R console is:

$$r = \frac{\sum (x - m_x)(y - m_y)}{\sqrt{\sum (x - m_x)^2 \sum (y - m_y)^2}}$$

Where,

x and y are two vectors of length 'n';  $m_x$  corresponds to means of x variable;  $m_y$  corresponds to means of y variable

The correlation between two variables can be estimated using correlation coefficients, which ranges between -1 to +1. The coefficient -1 indicates negative relationship between the variables, while +1 indicates positive relationship between the variables. The strength of correlation between the variables is estimated using the values of coefficients as follows (Singh and Narayana, 1993):

0 to 0.2 – weak correlation, the relation between the variables may not be often significant

0.2 to 0.45 – medium correlation, strong linearity in movement of variables exists but there are exceptions

>0.45 – strong relationship between the variables

1 – perfect correlation

To test the significance of correlation coefficients, the estimated values were compared with the table values of correlation coefficients (Fisher and Yates, 1963) at 5 per cent of significance with (n-2) degrees of freedom where 'n' is the number of genotypes used in the experiment.

**Stepwise multiple regression:** It is statistical measurement used to determine the important variables that contribute or show their influence to/on the response variable given by Draper and Smith, (1998). The best regression model from all the possible regressions involves the comparison of models based on the coefficient of determination ( $R^2$ ), adjust- $R^2$  and Mallows' Cp-statistic.

**Coefficient of determination ( $R^2$ ):** It shows the percentage of variations of response variable which is explained by the influence of the predictor variables involved in the regression model. It is estimated by using the formula

$$R^2 = \frac{SS_R}{SS_Y}$$



**Adjusted-(R<sup>2</sup>):** During the calculation of coefficient of determination, attention should be given for how many number of independent variables and sample size was involved. This is achieved by calculating adjusted coefficient of determination with formula

$$Adj.R^2 = 1 - \left[ \frac{n-1}{n-k-1} \right]^{(1-R^2)}$$

where,

n = size of the sample

k = number of independent variables

variables as possible. Regression analysis was conducted in STAR v 2.0.1 (Statistical Tools for Agricultural Research) software (IRRI, 2013).

**RESULTS AND DISCUSSION**

**Measures of variability:** Simple measures of variability that include mean, range and standard deviation were given in (Table 1). Seed yield resulted with mean 9.44, ranging from 1.99 to 34.38 with SD 5.24. Other traits, number of primary capsules/plant, number of seeds/primary capsule and 100 seed weight have shown SD as 2.16, 11.37 and 0.67 along with ranges 3 to 15, 5.89 to 69.89 and 1.97 to 6.00 respectively similar to the results reported by Omidi *et al.* (2009); Majidi and Zadhoush, (2014) and Kiran *et al.* (2017).

**Table 1. Mean, Range (min-max) and SD (standard deviation) estimates of seven traits in the RIL population.**

	DFF	DM	PH	NPCP	NSP	HSW	SY
mean	82.08	112.15	75.62	6.71	28.96	3.29	9.44
min	75.00	105.00	47.00	3.00	5.89	1.97	1.99
max	89.00	120.00	100.00	15.00	69.89	6.00	34.38
SD	3.20	3.26	9.34	2.16	11.37	0.67	5.24

DFF-Days to 50% flowering, DM Days to maturity, PH-Plant height, NPCP-Number of primary capitula/ plant, NSP-Number of seeds/ primary capitulum, HSW-100 seed weight, SY-Seed yield/ plant.

**Mallows Cp:** Cp represents the Mallows’s Cp, which is a metric used to pick the best regression model among different available models. This selection technique of regression model was given by Mallows, 1973 as follows:

$$Mallows\ Cp = \frac{RSS(p)}{S^2} - (n - 2p)$$

Where,

n = size of the sample

p = number of covariates

RSS (p) = residual sum of squares of the model containing p parameters

S<sup>2</sup> = mean residual sum of squares of the model containing all possible covariates

A model is said to be good fit according to the Cp criterion, if Cp d” p. If the value of Cp is closer or smaller than the value of p (number of independent variables in the model), the model has achieved the benefit of considering ‘good’ model containing as few

**Correlation analysis:** Knowledge of association between yield and its components determines the direction and number of traits to be considered for improvement of the crop by targeting selected characters. The correlation coefficients and the relation within and among dependent (yield) and independent (other related traits) variables, distribution of treatments with scatterplots, histogram and correlation significances between the variables can be seen in Figure 1.

In the above plot, distribution of each variable is shown in the diagonal, while below this represents the bivariate scatterplots with a fitted line. The bivariate scatterplot between days to 50% flowering and days to maturity has shown the strong positive relation as the points were scattered on the fit line in straight manner. The scatterplot between seed yield and 100 seed weight has shown weaker positive relation as the points were scattered in large amount around the fit line, while seed yield with days to 50% flowering and days to maturity showed no significant relation as the points scattered randomly around the fit line.

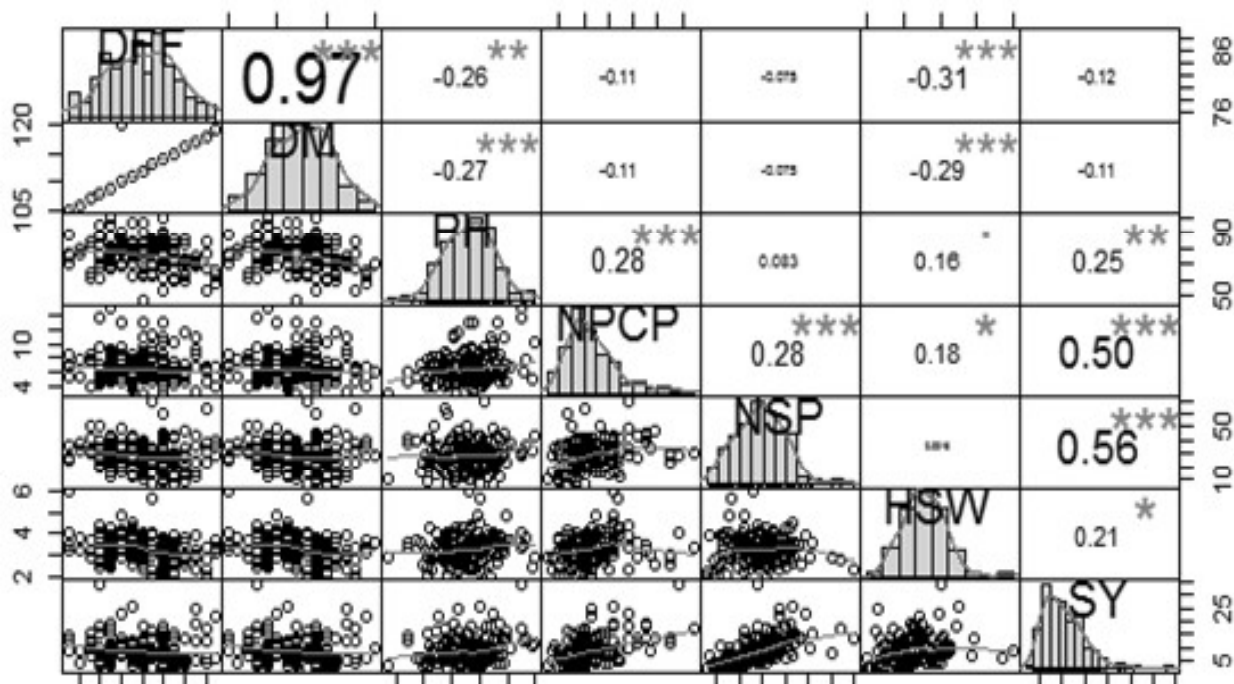


Figure 1. Plot showing distribution of variables, scatterplots and correlation with significance

**Correlation of seed yield with different traits:** Plant height, number of primary capitula/plant, number of seeds/primary capitulum and 100 seed weight were found to be correlated positively with yield indicating that selection practiced in these traits would improve the yield indirectly which was also observed by Pavithra *et al.* (2016). Association of number of primary capitula/ plant with seed yield was also observed by Jadhav *et al.* (2018) and Joseph Raju *et al.* (2019). Number of seeds/ primary capitulum was highly correlated with seed yield than any other trait. Similar observation was reported by Jadhav *et al.* (2018). The 100 seed weight showed positive correlation with seed yield as reported by Joseph Raju *et al.*, (2019) and Dhage *et al.* (2020). Plant height also correlated positively with seed yield as observed by Jadhav *et al.*, (2018) and Dhage *et al.*, (2020). With increase in plant height, there may be a chance of increase in number of nodes which would proportionally increase the number of branches, thereby would directly improve the number of primary capitula/ plant and number of seeds. Days to 50% flowering and days to maturity did not show any significant correlation but indicated negative relationship with seed yield as observed by Pavithra *et al.* (2016) and Joseph Raju *et al.* (2019).

**Correlation among different traits:** Days to 50% flowering was positively correlated with days to maturity as observed by Pavithra *et al.* (2016), Jadhav *et al.*,

(2018) and Dhage *et al.* (2020); negatively with plant height and 100 seed weight (Bidgoli *et al.*, 2006; Joseph Raju *et al.*, 2019 and Dhage *et al.*, 2020). Days to maturity did not show significant correlation but indicated negative association with plant height and 100 seed weight as observed by Kemal and Hailu, (2019). Plant height correlated positively with number of primary capitula/plant as observed by Bidgoli *et al.* (2006); Mozaffari and Asadi, (2006). Number of primary capitulum/plant was positively correlated with number of seeds/primary capitulum (Rathod *et al.*, 2021) and 100 seed weight (Jadhav *et al.*, 2018 and Rathod *et al.*, 2021). Number of seeds/primary capitulum showed no correlation with 100 seed weight, which was in contrary with the results by Rathod *et al.* (2021).

**Regression analysis:** Regression analysis helps us to estimate the relationship between the response variable and the influence of independent variables on the response variable, which is seed yield in the present study. The following equation is the best fitted linear model developed through stepwise regression analysis.

$$SY = -9.55 + 0.06PH + 0.77NPCP + 0.21NSP + 1.06HSW$$

The other characters were not included in model as the relative contribution was low compared to the predictor variables in the equation. The variation

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explained from the best fit predicted equation was 47% (Table 2) and the remaining unexplained 53% of the variation was not known or may be due to other traits which were not under consideration (Bidgoli *et al.*, 2006 and Łopatyńska *et al.*, 2021) while in contrast Yassein *et al.* (2020) explained 99.2% of variation. However, in case of strong linear relationship between seed yield and independent variables, this model can predict seed yield with high accuracy (Abdipour *et al.*, 2019). The model was positively correlated with seed yield with

can be estimated using  $R^2$  values,  $R^2$  – Adjusted values or Mallows' Cp. The most commonly used criterion was  $R^2$  for choosing between the alternative models (Schumacker, 1994; Diao *et al.*, 2015).

The  $R^2$  values (Table 4) for the models, have shown increase with increase of the number in predictor variables. But there is a sudden increase from 83.44 to 86.40 from one variable to two variable model, which indicates that the two variable model- NPCP + NSP

**Table 2. Summary of stepwise regression analysis and indices for predicting seed yield.**

RMSE	SY mean	CV (%)	$R^2$	$R^2$ -adjusted
3.88	9.44	55.68	0.47	0.45

**Table 3. Regression coefficients of the variables in the best fit model**

Variable	Regression coefficient	Standard error	t-value	p-value
Intercept	-9.55	2.93	-3.27	0.0014
PH	0.06	0.04	1.54	0.1263
NPCP	0.77	0.16	4.73	0.0000
NSP	0.21	0.03	7.29	0.0000
HSW	1.06	0.49	2.16	0.0324

$R^2$ -adjusted (0.45) from (table 2) indicating that 45% of seed yield variance was predictable from those four independent variables as observed by Golkar *et al.* (2011). The p-values indicates whether the relationship observed in the sample exists in the larger population or not which were represented in (table 3). The analysis showed that the variables, number of primary capitula/ plant, number of seeds/primary capitulum and 100 seed weight are statistically significant as they are showing p-values less than 0.05.

**Subset regression selection:** From all the predicted possible models, the best model to fit for regression

can be estimated to be the best regression fit without any bias. When all the predictor variables under consideration were included, the total variation explained was 87.47%, and the remaining 12.53% was unexplained. However, the adj.  $R^2$  has increased with the addition of plant height in the four predictor model from 86.96 to 87.01. This visualizes the improvement of the response variable with the inclusion of plant height in the selection indices along with number of primary capitula/ plant, number of seeds/ primary capitulum, 100 seed weight (Bahmankar *et al.*, 2014).

**Table 4. All possible prediction models for the response variable (seed yield)**

Model	C(p)	$R^2$	Adj. $R^2$
NSP	41.361	83.44	83.32
NPCP + NSP	10.04	86.40	86.21
DFF + NPCP+ NSP	6.56	86.89	86.61
DM + NPCP+ NSP+ HSW	3.74	87.31	86.96
PH+ NPCP + NSP + HSW	4.18	87.45	87.01
DFF + DM +PH + NPCP +NSP + HSW	6.00	87.47	86.94

DFF-Days to 50% flowering, DM Days to maturity, PH-Plant height, NPCP-Number of primary capitula/ plant, NSP-Number of seeds/ primary capitulum, HSW-100 seed weight, SY-Seed yield/ plant.

The C(p) values from the table 4 helps to pose an important balance with the number of independent variables and unbiased in estimating regression coefficients (Diaa *et al.*, 2015). Based on Mallow's Cp values, the four variable model DM + NPCP + NSP+ HSW has shown low Cp value, 3.74; similarly other four variable model, PH+ NPCP + NSP + HSW has also shown low as observed by Yassein *et al.* (2020). These values were closest to the number of independent variables and constant indicating that these models can be predicted to be precisely best fit without any bias for the enhancement of yield by using these variables as the selection indices (Abd El-Mohsen, 2013). From both the predictions, the predictor variables, PH, NPCP, NSP and HSW were showing positive relation with seed yield and can be used as selection indices for the yield improvement in the crop.

## CONCLUSION

Correlation analysis in the RIL population developed from the cross, CO-1 x EC-523368-2 revealed positive correlation of seed yield with plant height, number of primary capitula/plant, number of seeds/primary capitulum and 100 seed weight in safflower. Regression analysis revealed the important predictor variables namely, plant height, number of primary capitula/plant, number of seeds/primary capitulum and 100 seed weight using Mallows' C(p) estimate, R<sup>2</sup> and adj. R<sup>2</sup> coefficients. From both the results, it can be concluded that appropriate breeding strategies should be adopted for selection of these traits which may help to improve the seed yield potential in safflower.

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## CORRELATION AND PRINCIPAL COMPONENT ANALYSIS FOR YIELD-RELATED AND PHYSIOLOGICAL TRAITS UNDER HEAT STRESS IN GROUNDNUT (*Arachis hypogaea* L.)

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### ABSTRACT

The present investigation was carried out to study the relationship between yield-related and physiological traits under heat stress in groundnut. This experiment was conducted with a set of sixty-four groundnut genotypes at two dates of sowing during summer 2020-21 at International crops research institute for semi-arid tropics (ICRISAT), Patancheru, India. Observations were recorded on eleven traits days to 50% flowering, plant height, no of primary branches, pod yield per plant, kernel yield per plant, hundred kernel weight, shelling percentage, total biomass, harvest index, SPAD chlorophyll meter reading and membrane thermo-stability. The results of correlation analysis revealed that pod yield was positively associated with kernel yield, harvest index, biomass and SPAD chlorophyll meter reading in both environments, suggesting their role indirect selection for improvement in yield. Principal component analysis showed that four principal components explained the maximum variability of 67.57% and 68.52% in first date of sowing ( $S_1$ ) and second date of sowing ( $S_2$ ) environments respectively. Kernel yield per plant, pod yield per plant, and harvest index contributed for maximum variation of 26% with first principal component in both environments. These traits are significant to identify/develop heat-resilient varieties, as they contributed maximum towards the divergence of groundnut genotypes.

**KEYWORDS:** Correlation, groundnut, physiological traits, principal components, yield

Groundnut is a major source of oil, food and feed across the globe. It is grown in an area of 31.56 million hectares with a total production of 53.63 million metric tons (FAOSTAT, 2020). The kernels are comprised of oil (35-56 %), protein (25.9-32.4 %) and contain several beneficial minerals and vitamins (Akram *et al.*, 2018). Groundnut oil contains different fatty acids, including 80 % unsaturated fatty acids (UFA) and 20 % saturated fatty acids (SFA) (Wang *et al.*, 2015). Oleic (C18:1), linoleic (C18:2), palmitic (C16:0), and stearic (C18:0) are the major fatty acids. Groundnut oil is also a good source of plant sterols, particularly  $\beta$ -sitosterol, which has anti-cancer characteristics (Awad and Fink, 2000); antioxidants, vitamin E, and resveratrol, which fight free radicals, stress, and maintain cell membrane integrity (Meredith and Alfred, 2003). Along with the benefits listed above, groundnut oil with a high proportion of oleic acid and a low proportion of linoleic acid (high oleic/linoleic acid ratio) is preferred because it lowers the risk of cardiovascular disease (CVD) by lowering blood levels of low-density lipoproteins (LDL), has high oxidative stability, and is low in saturated fat (Wang and Hu, 2017).

Groundnut is affected by abiotic stresses like drought and heat, which are the major production constraints for the major groundnut growing regions of the world. The Intergovernmental Panel on Climate Change (IPCC) approximates that the global ambient temperature would rise by 1.5°C during 2030 to 2052 (IPCC, 2018). The optimum temperature for groundnut growth is between 25°C to 30°C but the pod yields can be substantially reduced if temperatures exceed 33°C (Prasad *et al.*, 2003). Reproductive phase is the most affected stage and the affected process is pollen grain development (Prasad *et al.*, 2001). In addition to yield, heat stress alters the concentration of oil, protein, sugars and fatty acids by preventing the enzymatic process required for their bio-synthesis, thereby hampers the nutritional quality of food legumes (Kaushal *et al.*, 2013; Sharma *et al.*, 2016; Haung *et al.*, 2019). Heat stress can be mitigated by adopting several morphological and physiological approaches to develop plants with greater heat tolerance. Physiological screening strategies for heat tolerance include cell membrane stability (Singh *et al.*, 2016; Chakraborty *et al.*, 2018) and chlorophyll content

(Burke, 2007). High temperatures alter membrane fluidity, permeability, and stability, and electrolyte loss caused by heat-induced cell membrane leakage is used to assess stress-related cellular damage in groundnut (Craufurd *et al.*, 2003). Under heat stress, photosynthesis can be impaired, owing to damage to photo-system II components in the chloroplast's thylakoid membranes and membrane proteins (Al-khatib and Paulsen, 1984).

Yield is a complex trait governed by several genes that is impacted by environmental and edaphic factors (Akinyosoye *et al.*, 2018). Knowledge of correlations among yield and its related traits is critical for yield improvement (Vidya and Oommen, 2002). When yield characters have a positive association, it is easier to improve yield. As crop species vary in yield and grain quality, Principal Component Analysis (PCA) can be used to uncover trends and minimise redundancy in data sets (Maji and Shaibu, 2012). Principal components are generally estimated either from correlation matrix or covariance matrix. It accomplishes this reduction by identifying directions (positive & negative), called principal components, along which the variation in the data is maximal (Singh and Narayanan, 1993). Cultivars adapted to high temperatures would have to be produced to maintain high yields in a progressively hotter climate. Given the likelihood that heat stress effects may become more apparent in the future, developing climate-resilient cultivars with high yield and desirable features will be critical. As a result, the aim of this study was to look into the relationship between yield-related and physiological features in groundnut.

## MATERIAL AND METHODS

### Plant material

A field experiment was conducted with sixty-four (64) advanced breeding lines of groundnut including three heat-tolerant checks (ICGV 02266, ICGV 03042, and ICGV 06424) from previous heat stress trials (Akbar *et al.*, 2017). The experiment was laid out in alpha lattice design with two replications with a spacing of 30 x 10 cm between rows and plants respectively during 2020-2021 at ICRISAT, Hyderabad. To examine the genotype by temperature interactions, a staggered date of sowing was adopted. The genotypes were sown at two different dates i.e., 1<sup>st</sup> February 2021 (S<sub>1</sub>) and 26<sup>th</sup> February 2021 (S<sub>2</sub>)

with 25 days intervals. The second sowing was planned to coincide flowering stage with high temperature.

### Observations recorded

Days to 50% flowering (DFF) was recorded by counting the number of days from sowing to when 50% of the total plant stand had reached flowering. From ten randomly sampled plants, Plant height (PH) was measured from the soil surface to the tip of main stem and number of primary branches (PB) was recorded as average number of primary branches. The chlorophyll content in the leaves was recorded between 8:00 to 9:30 am from fully expanded third leaf from the top during the flowering stage. The readings were recorded using Minolta SCMR-502 m (Tokyo, Japan) and the mean was calculated to give Soil plant analysis development (SPAD) chlorophyll meter reading (SCMR) (Rao *et al.*, 2001). Membrane thermostability (MT) was tested by exposing leaves to high temperature and computing relative injury to the membranes in terms of electrolytes leakage (Leopold *et al.*, 1981). Pod yield per plant (PY) was recorded as the average pod weight of ten random sample plants in grams. Kernel yield per plant (KY) was recorded as the average weight of the kernels after shelling of ten random sample plants in grams. Hundred kernel weight (HKW) was recorded as the weight of 100 kernels in grams. Shelling percentage (SH) was estimated as the proportion of shelled seed weight to the total weight of unshelled pods in %. Total biomass (BM) was recorded as the average total biomass weight of ten sample plants during the physiological maturity. Harvest index (HI) was calculated as the ratio of seed yield to the total biomass (Mukhtar *et al.*, 2013). Standard agronomic practices were followed; irrigation was given soon after planting and subsequently whenever required.

### Statistical analysis

Karl Pearson's correlation coefficient and principal component analysis between traits was calculated using SAS v9.4 (SAS Institute, 2018).

## RESULTS AND DISCUSSION

### Correlation analysis

Correlation analysis provides information about relationship among the various characters and determines the component characters, on which



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selection can be based for genetic improvement in the yield. The association among the yield-related and physiological traits in the environments is provided in Table 1.

KY with HI (0.76\*\*), BM (0.3\*) and SH (0.26\*); PB with DFF (0.35\*\*); PH with HKW (0.3\*). Negative significant correlation was observed between BM with HI (-0.36\*\*); PB with HI (-0.3\*); PH with DFF

**Table 1. Trait associations among the yield-related and physiological traits across the environments under heat stress**

Traits	Env	DFF	HI	HKW	KY	MT	PB	PH	PY	SCMR	SH
BM	S1	0.24	-0.36 **	0.08	0.30 *	-0.06	0.22	-0.04	0.32 **	0.10	-0.05
	S2	0.31 *	-0.22	0.20	0.35 **	-0.04	0.29 *	0.06	0.33 **	0.10	0.14
	Pooled	0.31 *	-0.39 **	0.19	0.22	-0.06	0.34 **	-0.09	0.21	0.10	0.07
DFF	S1		-0.09	-0.17	0.07	-0.07	0.35 **	-0.34 **	0.15	0.03	-0.23
	S2		0.01	0.15	0.19	0.12	0.14	0.17	0.09	0.07	0.28 *
	Pooled		-0.14	0.00	0.05	-0.04	0.36 **	-0.11	0.07	0.05	0.00
HI	S1			0.15	0.76 **	0.16	-0.30 *	-0.05	0.7 **	0.03	0.24
	S2			-0.02	0.83 **	0.06	0.01	0.01	0.74 **	0.04	0.28 *
	Pooled			0.08	0.80 **	0.16	-0.19	-0.08	0.76 **	0.17	0.10
HKW	S1				0.22	0.13	-0.17	0.30 *	0.24	0.24	-0.12
	S2				0.11	0.19	-0.12	0.26 *	-0.04	0.16	0.38 **
	Pooled				0.21	0.28 *	-0.21	0.28 *	0.15	0.22	0.18
KY	S1					0.14	-0.13	-0.06	0.94 **	0.13	0.26 *
	S2					0.06	0.17	0.06	0.91 **	0.10	0.35 **
	Pooled					0.12	0.02	-0.15	0.95 **	0.26 *	0.16
MT	S1						-0.17	0.17	0.11	0.13	0.13
	S2						-0.28 *	0.26 *	0.04	-0.09	0.03
	Pooled						-0.30 *	0.19	0.12	-0.01	0.03
PB	S1							-0.33 **	-0.11	0.09	-0.05
	S2							-0.02	0.26 *	0.10	-0.16
	Pooled							-0.28 *	0.06	0.14	-0.15
PH	S1								-0.08	0.04	0.05
	S2								-0.05	-0.19	0.21
	Pooled								-0.19	-0.20	0.11
PY	S1									0.13	-0.08
	S2									0.12	-0.07
	Pooled									0.29 *	-0.14
SCMR	S1										0.00
	S2										-0.06
	Pooled										-0.11

\*P < 0.05; \*\* P < 0.01; \*\*\*P < 0.001; ns non-significant

**Note:** S<sub>1</sub>= First date of sowing; S<sub>2</sub>= Second date of sowing; DFF= Days to 50% flowering; PH= plant height; PB= no. of primary branches; PY= Pod yield per plant; KY=Kernel yield per plant; HKW= hundred kernel weight; SH= Shelling percentage; BM= Biomass; HI= Harvest index; SCMR= SPAD chlorophyll meter reading; MT= Membrane thermo-stability

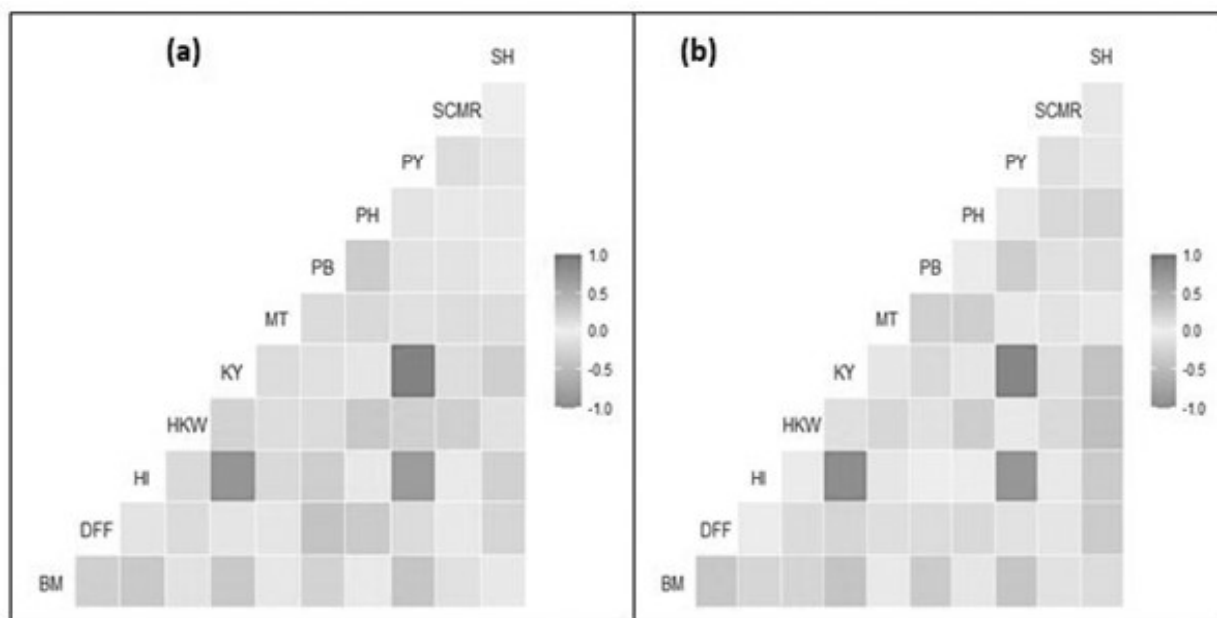
In S<sub>1</sub>, the correlations among the yield-related and physiological traits are provided in Figure 1a. Significant positive correlations were observed between traits, PY with KY (0.94\*\*), HI (0.7\*) and BM (0.32\*);

(-0.34\*\*) and PB (-0.33\*\*). SCMR and MT showed non-significant correlations with yield related traits.

In S<sub>2</sub>, the significant positive correlations were recorded between the traits, PY with KY (0.91\*\*), HI

(0.74\*\*), BM (0.33\*\*) and PB (0.26\*); KY with HI (0.83\*\*), SH (0.35\*\*) and BM (0.35\*\*); HKW with SH (0.38\*\*) and PH (0.26\*); SH with HI (0.28\*) and DFF (0.28\*); BM with DFF (0.31\*) and PB (0.29\*); MT with PH (0.26\*). Negative and significant correlation was observed between MT and PB (-0.28\*) (Figure 1b).

(Bagathariya and Patel, 2017), SCMR (Sab *et al.*, 2018) and MT in wheat (Islam *et al.*, 2017). The positive correlation of SH with HKW (Tirkey *et al.*, 2018) and HI (Roy *et al.*, 2021) were reported in groundnut. Negative correlation was observed between BM and HI. These associations are in consistent with earlier



**Note:** DFF= Days to 50% flowering; PH= plant height; PB= no. of primary branches; PY= Pod yield per plant; KY=Kernel yield per plant; HKW= hundred kernel weight; SH= Shelling percentage; BM= Biomass; HI= Harvest index; SCMR= SPAD chlorophyll meter reading; MT= Membrane thermo-stability

**Figure 1.** Correlogram showing the association among the assessed traits for sixty-four groundnut genotypes under heat stress conditions (a)  $S_1$  (first date of sowing) (b)  $S_2$  (second date of sowing) evaluated during 2020–2021.

The correlations observed across the environments revealed positive significant associations of PY with KY (0.95\*\*), HI (0.76\*\*) and SCMR (0.29\*); KY with HI (0.8\*\*) and SCMR (0.26\*); HKW with MT (0.28\*) and PH (0.28\*); BM with DFF (0.31\*) and PB (0.34\*\*); PB with DFF (0.36\*\*). However, significant negative correlations were recorded for BM with HI (-0.39\*); PB with MT (-0.3\*) and PH (-0.28\*).

The common significant correlations observed among the yield-related and physiological traits include positive correlation of PY with KY, BM, HI, PB and SCMR. Similar reports were confirmed for correlation between PY with KY, BM and HI (Babariya and Dobariya, 2012; Kushwah *et al.*, 2016), PB (John *et al.*, 2019) and SCMR (John *et al.*, 2007; Rao, 2016; Sab *et al.*, 2018) in groundnut. Earlier reports in groundnut confirmed the positive correlation of KY with PY (Thirumala Rao *et al.*, 2014), SH (Zongo *et al.*, 2017), HI (Trivikrama Reddy *et al.*, 2017), BM

reports by Ghassemi-Golezani *et al.*, (2012) in maize and Karim *et al.*, (2020) in groundnut. The negative association of DFF with PH was reported by Babariya and Dobariya (2012). The present results on correlation coefficients revealed that kernel yield per plant, number of primary branches, biomass, harvest index and SPAD chlorophyll meter reading were the most important attributes that contributes towards higher pod yield. The interrelationship among yield components and physiological traits would help in increasing the yield levels and these traits could be considered for selection in groundnut breeding.

#### Principal component analysis

Principal component analysis was performed for eleven traits among sixty-four groundnut genotypes. The results revealed four principal components (PCs) with eigenvalues greater than one under  $S_1$  and  $S_2$  environments, respectively (Table 2). The four principal components with eigen value >1 accounted for 67.57%

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and 68.52% of the total phenotypic variation under  $S_1$  and  $S_2$  environments, respectively (Table 2). Under  $S_1$  environment, the highest coefficient in PC1 was

the primary source of variation followed by PB (0.43) and BM (0.39). PC2 accounted for 18.16% of total variation and was positively loaded with DFF, PB,

**Table 2. Eigen values and variance of yield-related and physiological traits among sixty-four groundnut genotypes evaluated under heat stress conditions**

Principal Components	Eigen values	S1		S2		Cumulative Percentage of variance
		Percentage of variance	Cummulative percentage of variance	Eigen Values	Percentage of variance	
1	2.87	26.05	26.05	2.93	26.62	26.62
2	2.00	18.16	44.22	1.88	17.12	43.73
3	1.47	13.32	57.54	1.61	14.65	58.39
4	1.10	10.03	67.57	1.11	10.13	68.52
5	0.99	9.03	76.61	1.00	9.05	77.57
6	0.86	7.83	84.44	0.79	7.20	84.77
7	0.62	5.65	90.09	0.75	6.78	91.56
8	0.57	5.19	95.28	0.47	4.23	95.79
9	0.49	4.47	99.76	0.45	4.10	99.89
10	0.02	0.22	99.97	0.01	0.08	99.97
11	0.00	0.03	100.00	0.00	0.03	100.00

**Note:**  $S_1$ = First date of sowing;  $S_2$ = Second date of sowing

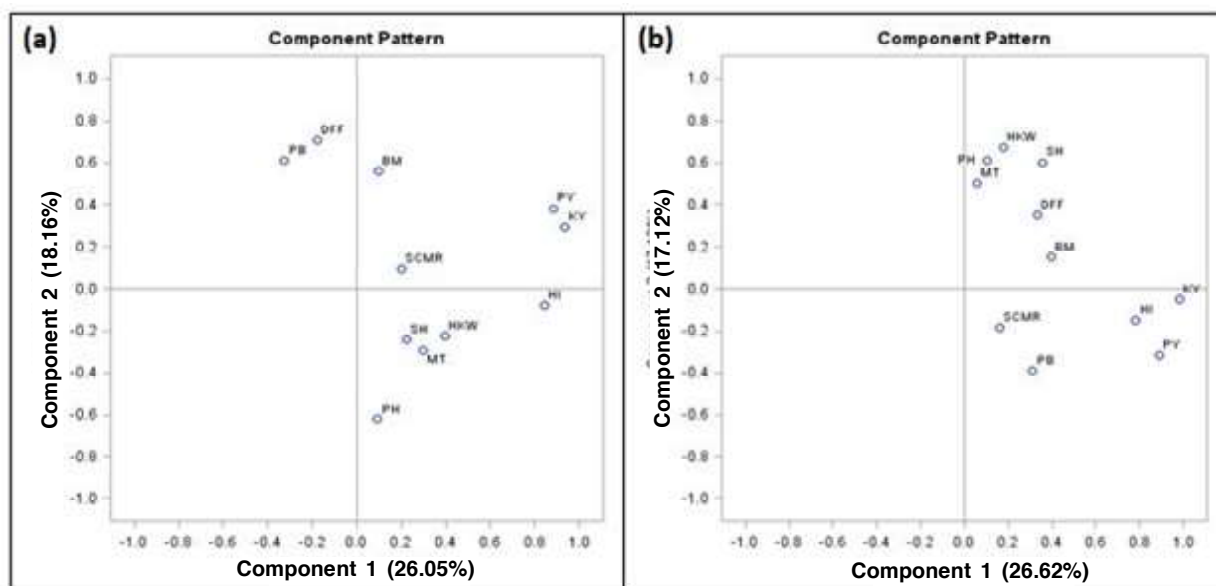
observed in KY (0.55), PY (0.52) and HI (0.50). PC1 was accounted for 26.05% of total variation and positively loaded with PH, PY, KY, HKW, SH, BM, HI, MT and SCMR and negatively loaded with DFF and PB (Table 3; Figure 2). In the PC2, DFF (0.50) was

PY, KY, BM and SCMR and negatively loaded with PH, HKW, SH, HI and MT. In PC3, the highest coefficient was observed for HKW (0.52) and BM (0.43). PC3 explained for 13.32% of total variation and was loaded positively with PH, PB, PY, HKW, BM, MT

**Table 3. Principal component scores of yield-related and physiological traits among sixty-four groundnut genotypes evaluated under heat stress conditions**

Traits	$S_1$				$S_2$			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
DFF	-0.11	0.50	-0.03	0.14	0.19	0.26	0.36	-0.16
PH	0.06	-0.44	0.38	-0.01	0.06	0.44	-0.02	-0.41
PB	-0.19	0.43	0.03	0.33	0.18	-0.28	0.45	-0.27
PY	0.52	0.27	0.04	-0.17	0.52	-0.23	-0.08	-0.09
KY	0.55	0.21	-0.06	0.07	0.57	-0.04	-0.10	0.00
HKW	0.23	-0.16	0.52	-0.16	0.10	0.49	0.12	0.41
SH	0.13	-0.17	-0.32	0.72	0.21	0.44	-0.03	0.19
BM	0.06	0.39	0.43	0.23	0.23	0.11	0.56	-0.11
HI	0.50	-0.06	-0.35	-0.13	0.46	-0.11	-0.44	0.06
MT	0.18	-0.21	0.11	0.33	0.03	0.36	-0.27	-0.12
SCMR	0.12	0.07	0.40	0.33	0.10	-0.14	0.24	0.70

**Note:**  $S_1$ = First date of sowing;  $S_2$ = Second date of sowing; PC1= principal component 1; PC2= principal component 2; PC3= principal component 3; PC4= principal component 4; DFF= Days to 50% flowering; PH=plant height; PB= no. of primary branches; PY= Pod yield per plant; KY=Kernel yield per plant; HKW= hundred kernel weight; SH= Shelling percentage; BM= Biomass; HI= Harvest index; SCMR= SPAD chlorophyll meter reading; MT= Membrane thermo-stability



**Note:** DFF= Days to 50% flowering; PH= plant height; PB= no. of primary branches; PY= Pod yield per plant; KY=Kernel yield per plant; HKW= hundred kernel weight; SH= Shelling percentage; BM= Biomass; HI= Harvest index; SCMR= SPAD chlorophyll meter reading; MT= Membrane thermo-stability

**Figure 2.** Principal components biplot showing the relationship between assessed traits among sixty-four groundnut genotypes under heat stress conditions (a) S<sub>1</sub> (first date of sowing) (b) S<sub>2</sub> (second date of sowing) evaluated during 2020–2021.

and SCMR and negatively with DFF, KY, SH and HI. In PC4, the largest coefficient was observed for SH (0.72), followed by MT (0.33), SCMR (0.33) and PB (0.33). PC4 showed 10.03% of total variation and was positively loaded with PB, KY, SH, BM, MT and SCMR and negatively with DFF, PH, PY, HKW and HI.

Under S<sub>2</sub>, the primary source of variation in the PC1 was observed in KY (0.57), PY (0.52) and HI (0.46). PC1 accounted for 26.62% of total variation and showed positive loadings with all the traits. For PC2, HKW (0.49) contributed highest variation followed by PH (0.44) and SH (0.44). PC2 showed 17.12% of total variation and was positively loaded with DFF, PH, HKW, SH, BM and MT, whereas negatively with PB, PY, KY, HI and SCMR. The largest coefficient in PC3 was exhibited by BM (0.56), PB (0.45) and DFF (0.36). PC3 explained 14.65% of total variation and was positively loaded with DFF, PB, HKW, BM and SCMR, whereas negatively loaded with PH, PY, KY, SH, HI and MT. For PC4, the largest coefficient was observed for SCMR (0.70) and HKW (0.41). PC4 explained 10.13% of total variation and was positively loaded with HKW, SH, HI and SCMR, while negatively with DFF, PH, PB, PY, BM and MT.

PCA was generally used to reduce the large number of observed characteristics into smaller number

that have maximum contribution in discriminating the genotypes. The results exhibited four main components in both S<sub>1</sub> and S<sub>2</sub> environments with a variation of 67.57% and 68.52% respectively. Under S<sub>1</sub> environment, traits like Kernel yield per plant, pod yield per plant, and harvest index associated with PC1 and contributed a variation of 26.05%. Whereas, under S<sub>2</sub> environment, traits kernel yield per plant, pod yield per plant, and harvest index associated with PC1 and contributed a variation of 26.62%. The traits associated with these main components are important as they contributed maximum towards divergence of groundnut genotypes and can be further useful in breeding programs.

## CONCLUSION

Selection on the basis of association between yield-related and physiological traits is critical for identifying the key characters, which can be exploited in further breeding programme. Correlation analysis conclude that pod in groundnut can be improved by focusing on the traits like biomass, hundred kernel weight and SPAD chlorophyll meter reading under heat stress, suggesting their use as the selection criteria for the improvement of yield in groundnut. Principle component analysis revealed that pod yield, kernel yield and harvest index associated with first component

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contributed for maximum variation, suggesting these traits have much influence during selection and development of heat-resilient varieties in groundnut.

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## ASSESSMENT OF BLAST DISEASE INCIDENCE IN MAJOR RICE GROWING AREAS OF TELANGANA STATE

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### ABSTRACT

A survey on incidence of rice blast disease was conducted in 13 major rice growing districts of Telangana. Percent disease incidence was calculated as the proportion of plants showing symptoms, out of the total number of plants. The incidence varied from 8.24 per cent in Vikarabad district to 58.26 per cent in Warangal district. Disease incidence was recorded among all the prominent cultivars grown in their particular regions and it was observed that different cultivars showed different levels of incidence irrespective of the regions. Among the different cultivars, HMT Sona showed the highest incidence of 71.33 per cent whereas cultivar Tellahamsa with 49.16 percent incidence. The maximum isolation rate of the pathogen *M. oryzae* was recorded from Siddipet with 75 per cent followed by Mahabubnagar with 50 per cent. The disease was observed from nursery to grain hardening stage. The study of disease incidence assessment helps in selecting cultivars as well planning the blast management strategies in Telangana state.

**Keywords:** Rice blast, disease incidence, survey.

Rice [*Oryza sativa* L.] is a major staple food for half of the world's population. It is widely cultivated in India, China, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, Philippines and Brazil. China is the leading rice producer followed by India, Indonesia and Bangladesh. Rice provides 20 per cent of the world's dietary energy supply followed by maize and wheat. Rice contributes to a major proportion of dietary calories (21 % of energy and 15 % of protein) for the population in developing countries and is an important component of food security (Gnanam-anickam, 2009). In India, rice crop occupies an area of about 44.0 million hectares with a production of 121 million tonnes and productivity of 4120 kg ha<sup>-1</sup> (USDA, 2021). In Telangana, rice is cultivated in 27.72 lakh hectares with a production of 5.17 million tonnes (DES, 2020).

Rice diseases often act as biotic constraints limiting rice production. The crop suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is one of

the most destructive diseases due to its wide spread (over 80 countries) and potential to cause up to 75 per cent grain loss in India when conditions are favourable (Padmanaban, 1965). Rice blast caused by *Magnaporthe grisea* (Hebert, 1971) Barr [Anamorph: *Pyricularia grisea* (Cooke) Sacc.] is a filamentous ascomycetes fungus infecting more than 50 hosts. This disease is distributed in more than 85 countries around the world (Kato, 2001). In Brazil, it has been reported to cause a 100 per cent yield loss in the production in a newly released upland cultivar called Colaaso (Prabhu *et al.*, 2009). The yield loss caused by the blast disease has been estimated to be between 10 per cent and 30 per cent in many countries (Gurinder *et al.*, 2006). In India, this disease caused 15 per cent -20 per cent yield loss in rice production (Dibyendu *et al.*, 2017). Under favourable conditions, blast disease destroys the entire crop within 15-20 days (Ashkani *et al.*, 2015; Sakulkoo *et al.*, 2018; Wilson and Talbot, 2009). The 10 Per cent of yield lost due to blast disease is sufficient to feed 60 million people for a year (Scardaci *et al.*, 2003).



## ASSESSMENT OF BLAST DISEASE INCIDENCE IN MAJOR RICE GROWING AREAS

Occurrence of rice blast was first recorded in China 1637, later from Japan 1704, from Italy in 1828, and from USA in 1876. In India, the disease gained importance when a devastating epidemic occurred in Thanjavur (Tanjore) delta of Tamilnadu during 1918. In Telangana state, it was first identified in Nizamabad district (Nagarajan, 1988). However, intensity of the disease varies in different regions in different years. Rice blast, potentially a devastating disease results in yield loss as high as 70-80 per cent when predisposing factors such as high mean temperature values, degree of relative humidity higher than 85-89 per cent, presence of dew, drought stress and excessive nitrogen fertilization favour epidemic development. Hence the present study was conducted to get the primary information regarding the disease in the major rice growing areas of Telangana.

### MATERIAL AND METHODS

A roving survey was conducted in different rice ecosystems, viz., irrigated, rainfed, tank fed, bore wells, low lying and upland ecosystems during *Rabi* 2019-20 in major rice growing districts viz., Nizamabad, Medak, Vikarabad, Karimnagar, Jagtial, Warangal, Mancherial, Siddipet, Rajanna Sircilla, Nirmal, Mahabubnagar, Jogulamba Gadwal and Suryapet of Telangana. Prominent rice growing areas were chosen for assessment of disease, per cent disease incidence (PDI), stage of the crop, variety grown, cropping pattern were recorded. Samples showing typical symptoms of blast disease were collected in butter paper covers to avoid saprophytic contamination, brought to the plant pathology laboratory of ICAR- Indian Institute of Rice Research and stored for further studies. Observations were recorded in four one-meter square areas randomly in each field by walking diagonally starting from South west corner. The disease in observed fields was expressed as per cent disease incidence (PDI).

$$\text{PDI} = \frac{\text{Diseased hills observed}}{\text{Total number of hills observed}} \times 100$$

### Isolation and identification of the pathogen

The pathogen was isolated by following standard tissue isolation procedure (Tuite, 1969). Small bits of diseased leaves were cut along with some healthy tissue with the help of a sterile scalpel and surface sterilized with one per cent sodium hypochlorite

solution for one minute and rinsed aseptically in three changes of sterilized distilled water. Such surface sterilized leaf bits were transferred aseptically into sterilized Petri dishes containing solidified oat meal agar medium and incubated at  $28 \pm 1^\circ\text{C}$  for 24 hours and checked for sporulation. The culture was transferred into sterilized Petri dishes containing solidified oat meal agar medium and incubated at  $28 \pm 1^\circ\text{C}$  for two weeks in a BOD incubator.

The copious nature of blast fungi in the sample was described by isolation rate (IR) and calculated as per Rajini *et al.*, (2019) with slight modifications as

$$\text{Isolation rate (IR)} = 100 \times \frac{\text{Total number of isolates yielded}}{\text{Total number of samples collected}}$$

### RESULTS AND DISCUSSION:

Among the 13 districts, highest mean per cent disease incidence was recorded in Warangal (PDI-58.26 %) followed by Siddipet (PDI-57.67 %), Karimnagar (PDI-47.26 %), Suryapet (PDI-43.88 %), Mahabubnagar (PDI-43.79 %) and Jogulamba gadwal (PDI-40.35 %) districts. The lowest mean disease incidence 8.24 per cent was recorded in Vikarabad district followed by Jagtial (PDI-18.88 %) and Nirmal (PDI-21.85 %) districts (Table-2).

The data presented in (Table-1) revealed that highest mean blast incidence 86.75 per cent was recorded in Khazipet mandal of Warangal district in a range of 82 per cent to 90 per cent followed by Kodhad mandal of Suryapet district with (75.50 % mean per cent incidence ranging of 66 % to 82 %), Geesugonda mandal (73.82 %) with a range of 65.62 per cent to 85.93 per cent of Warangal and Hanwada mandal (71.75 %) with a range of 62 per cent to 79 per cent of Mahbubnagar district. The lowest mean percent incidence (6.97 %) was noticed in Dharur mandal ranging from 4.61 per cent to 10.93 per cent followed by Dharmapuri mandal (7.08 % with 3.12 to 15.62 %), Yalal mandal (9.51 % with 2.77 % to 23.43 % range), Thimmapur (15.93 %) with 12.5 % to 20 % range) and Dusturabad (17.90 % with 8.1 % to 32.46 % range).

The results of the present study also indicated that different cultivars showed varied incidence levels irrespective of the location they were cultivated (Table-3). The highest disease mean incidence of 71.33 per cent was recorded in variety HMT Sona which was predominantly grown in Suryapet district followed by

**Table 1. Incidence of blast in different mandals of Telangana state**

District	Mandal	Range of percent disease incidence	Mean percent disease incidence
Nizamabad	Bodhan	4.68-36.00	19.57
	Rudrur	30.00-50.00	37.82
Medak	Chegunta	15.62-44.44	33.04
	Sankarampet	41.66-44.44	42.70
Vikarabad	Yalal	2.77-23.43	9.51
	Dharur	4.61-10.93	6.97
Karimnagar	Kothapally	25.26-75.78	50.31
	Ramadugu	54.73-67.36	60.78
	Burugupalli	53.33-71.57	62.03
	Thimmapur	12.50-20.00	15.93
Jagtial	Gollapally	12.50-64.06	30.69
	Dharmapuri	3.12-15.62	7.08
Manchiryal	Manchiryal	20.27-30.55	26.04
	Lakshettipet	37.50-57.81	48.48
Warangal	Khanapur	8.33-60.00	33.95
	Narsampet	9.72-90.00	41.02
	Chennaraopet	34.20-75.34	55.80
	Geesugonda	65.62-85.93	73.82
	Khazipet	82.00-90.00	86.75
Siddipet	Akkanapet	37.83-87.00	63.49
	Husnabad	40.32-70.31	51.86
Rajanna siricilla	Vemulawada	13.88-46.87	27.20
	Chandurthi	44.44-48.48	46.55
Nirmal	Dusturbad	8.10-32.46	17.90
	Kaddam	8.06-41.93	25.80
Mahabubnagar	Hanwada	62.00-79.00	71.75
	Koilkonda	50.00-55.00	52.75
Jogulamba Gadwal	Rajoli	43.75-66.00	54.65
	Itikyal	50.00-61.11	55.20
Suryapet	Kodhad	66.00-82.00	75.50
	Ananthagiri	13.88-64.00	42.60

Tellahamsa with mean disease incidence of 49.16 per cent, KNM 118 mean disease incidence of 45.87 per cent, MTU 1010 mean disease incidence of 44.64 % and JGL 2-44-23 mean disease incidence of 43.88 per cent. The ascending order of the remaining cultivars regarding the mean disease incidence was recorded as Sai Ram gold (6.04%) < Jai Sri Ram (24.32%) < Mitra plus (28.46%) < Annapurna (29%) < BPT 5204 (32.25%) < Nithya (30.31%) < RNR 15048 (36.11%) < MTU 1156 (36.26%) < MTU1153 (37.41%) and Devika gold (39.25%) (Figure 1).

A total of 144 diseased samples containing 100 leaf samples and 44 panicle samples collected

from 13 different districts of Telangana. From these 144 infected samples a total of 40 blast cultures were isolated out of which, 36 isolates were recovered from leaf samples and four isolates from panicle samples. The isolation rate (Table 4) of the samples collected from different districts was calculated and it was found that the maximum isolation rate was recorded from Siddipet with 75 per cent followed by Mahabubnagar with 50 per cent of the total similarly the isolation rate was 41.17 per cent from warangal and the lowest was recorded from Vikarabad, Medak and Nizamabad districts (Figure 2). The variation in isolation rate of the pathogen from the leaf and panicles can be attributed to the weather conditions, farmer's practices viz., prior

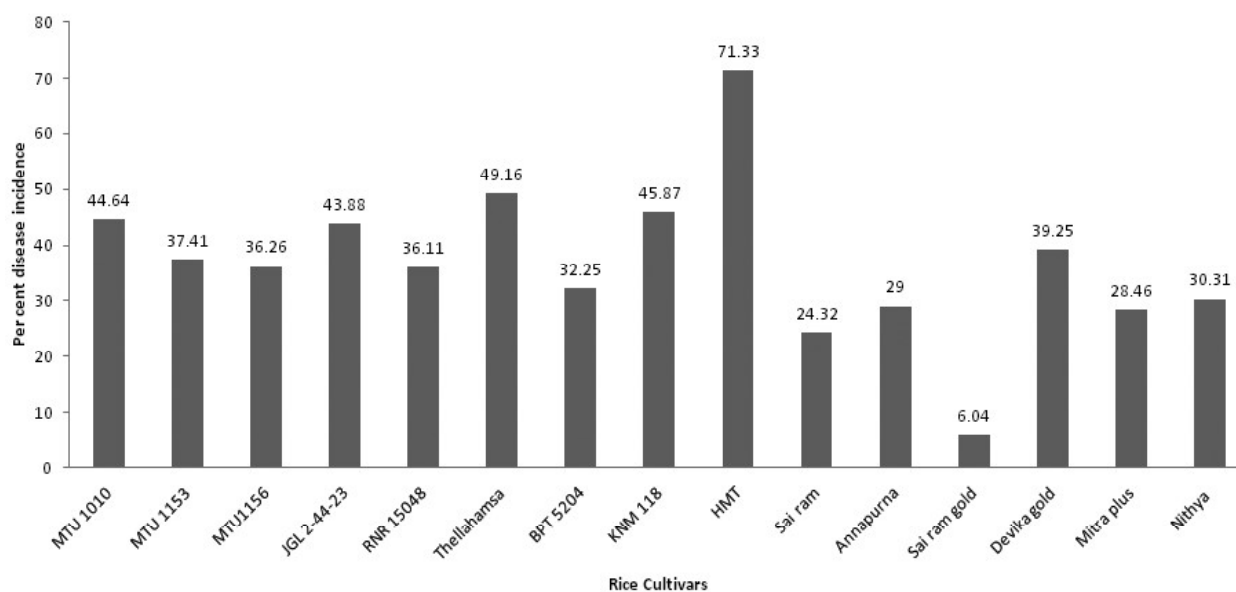
ASSESSMENT OF BLAST DISEASE INCIDENCE IN MAJOR RICE GROWING AREAS

**Table 2. Incidence of blast disease in major rice growing districts of Telangana**

District	Mean per cent disease incidence
Nizamabad	28.69
Medak	37.87
Vikarabad	8.24
Karimnagar	47.26
Jagtial	18.88
Mancherial	37.26
Warangal	58.26
Siddipet	57.67
Rajannasircilla	36.87
Nirmal	21.85
Mahabubnagar	43.79
Jogulambagadwal	40.35
Suryapet	43.88
<b>CD</b>	3.752
<b>CV</b>	6.018

**Table 3. Mean percent blast incidence among the rice cultivars grown in Telangana**

Variety	Mean per cent disease incidence
MTU 1010	44.64
MTU 1153	37.41
MTU1156	36.26
JGL 2-44-23	43.88
RNR 15048	36.11
Tellahamsa	49.16
BPT 5204	32.25
KNM 118	45.87
HMT Sona	71.33
Jai Sri ram	24.32
Annapurna	29.00
Sai ram gold	6.04
Devika gold	39.25
Mitra plus	28.46
Nithya	30.31



**Figure 1. Mean per cent blast incidence among rice cultivars grown in Telangana during Rabi 2019-20.**

application of fungicides, high amount of nitrogen applications, improper irrigation which might have affected the survival and spread of inoculum and which ultimately led to highly aggregated distribution in each cultivar among the fields.

The present results on the incidence of blast disease in different agro climatic zones of Telangana are in accordance with Hossain and Kulakarni, who conducted survey for rice blast during Kharif, 1999 in different villages of Dharwad, Belgaum and Uttara

Kannada districts of Karnataka and reported maximum disease incidence in Haliyal (61.66 %) and Mundagod (54.00 %) talukas of North Karnataka. Bhaskar *et al.*, (2018) conducted survey for rice blast during Kharif and Rabi seasons, 2015-16 in different districts of Andhra Pradesh and reported maximum disease incidence in Kovvur mandal of Nellore district. Higher intensity of leaf blast was observed in parts of Jagtial, Rajanna Sircilla of Northern Telangana and parts of Khammam and Ranga reddy whereas, neck blast was high in parts of Sangareddy (Production Oriented Survey, 2020).

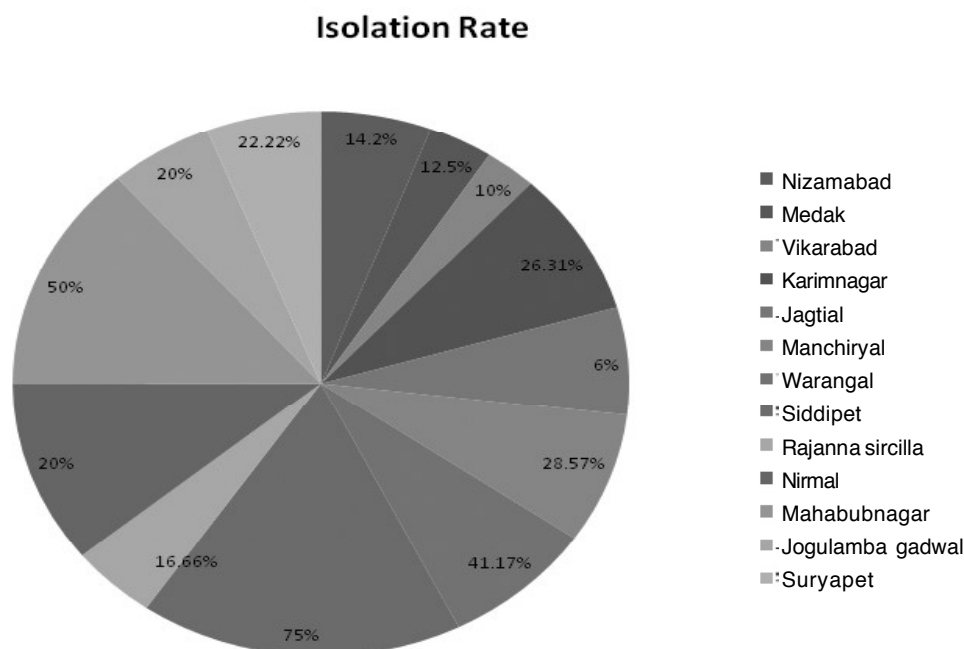
**Table 4. Isolation rate of the blast pathogen in major rice growing districts of Telangana**

District	Isolation rate in percent
Nizamabad	14.20
Medak	12.50
Vikarabad	10.00
Karimnagar	26.31
Jagityal	17.60
Manchiryal	28.57
Warangal	41.17
Siddipet	75.00
Rajannasircilla	16.66
Nirmal	20.00
Mehbubnagar	50.00
Jogulambagadwal	20.00
Suryapet	22.22

the major factor that changed in the enclosed blast nursery of the Dawei Mountain district over the three years. The climate in July and August was correlated closely with the occurrence of rice panicle blast. Correspondingly, the variation of rice field isolates should be highly correlated with the climate change. Hence in the present study, the occurrence of blast disease is ascertained to the change in the climate and might be due to positive correlation of conducive weather parameters like temperature, relative humidity and rainfall (Production Oriented Survey, 2020).

**CONCLUSION**

The variation in the disease incidence may be related to differences in variety grown, time of planting, fertilizer and weather conditions like relative humidity,



**Figure 2. Isolation rate of blast pathogen collected from different geographic locations of Telangana**

Zirong *et al.*, (2019) reported that the meteorological data showed the main difference in occurrence of blast disease between 2013 and the other two years (2014, 2015) was the weather during July and August. During these two months in 2013, the average temperature was near 30°C consistently, with low precipitation and average air humidity of only 68% caused very slight occurrence of rice panicle blast. In contrast, during 2014 and 2015, the climate was suitable and accelerated the occurrence of rice blast. Temperature and humidity in the Dawei Mountain in July to August in 2013 were significantly different from those in 2014 and 2015. Thus weather conditions were

low temperature and due to continuous rain on these locations during the period of survey.

In the present study, it was concluded that the disease incidence highly varied among the cultivars and regions in accordance to the weather conditions. Hence, the present study will be a limelight for further studies on host pathogen interaction in rice where development of resistance is dependent on the presence of corresponding *Avr* gene in *M. oryzae*. Thus knowledge of the genetic variation within and among pathogen population is an important component.

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Table 5. *M. oryzae* isolates collected from major rice growing areas of Telangana

Agro climatic zone/ State	District	Place of collection	Latitude	Longitude	Stage of the crop	Variety	Host origin	Isolate code
Northern Telangana Zone	Nirmal	Revojjipeta	19.076867	78.924455	Dough stage	Sairam gold	Leaf	Mo-1
		Kadem	19.093413	78.712855	Dough stage	BPT-5204	Leaf	Mo-2
	Mancheriyal	Talamadugu	19.095158	78.947727	Milky stage	BPT-5204	Neck	Mo-3
		Kothur	18.909807	79.119770	Panicle emergence	RNR- 15048	Leaf	Mo-4
	Nizamabad	Venkatapum	18.223422	77.582911	Milky stage	JGL-2 44 23	Leaf	Mo-5
		Achampalli	18.364552	77.492011	Dough stage	Jai sri ram	Neck	Mo-6
		RARS	18.345188	77.490941	Tillering	TN 1	Leaf	Mo-7
	Jagityal	Gunjapadu	18.778727	79.064535	Tillering	JGL 2 44 23	Leaf	Mo-8
		Rapalli	18.819982	79.045630	Tillering	MTU1153	Leaf	Mo-9
		Kothapet	19.022305	79.053808	Tillering	KNM 118	Leaf	Mo-10
		Jaina	19.009414	79.053736	Tillering	MTU 1153	Leaf	Mo-11
		Vemulavada	18.562110	78.778900	Tillering	MTU-1153	Leaf	Mo-12
	Rajannasircilla	Srirampally	18.619522	79.039018	Tillering	MTU1156	Leaf	Mo-13
		Asifnagar	18.453432	79.024112	Tillering	JGL 2 44 23	Leaf	Mo-14
Kurtyal		18.558113	78.997680	Tillering	MTU1153	Leaf	Mo-15	
Karimnagar	Gangadara	18.558162	78.998018	Tillering	MTU1156	Leaf	Mo-16	
	Thimmapur	18.251682	79.159788	Tillering	JGL 2 44 23	Leaf	Mo-17	
	Alugunur	18.175032	79.205462	Milky stage	Mitra	Neck	Mo-18	
	Rajpet	17.965162	79.931368	Tillering	MTU-1010	Leaf	Mo-19	
Warangal	Kothapally	17.986170	79.907202	Tillering	KNM-118	Leaf	Mo-20	
	Khannapur	17.950892	79.960080	Tillering	MTU-1010	Leaf	Mo-21	
	Chennaraopet	17.890694	79.875009	Tillering	MTU-1010	Leaf	Mo-22	
	Jirapally	17.986047	79.907000	Tillering	RNR-15048	Leaf	Mo-23	
	Akkanapet	18.073118	79.202399	Tillering	MTU 1010	Leaf	Mo-24	
Siddipet	Akkanapet	18.073118	79.202390	Panicle emergence	Annapurna	Leaf	Mo-25	
	Gouagakunta	18.037257	79.213537	Milky stage	MTU- 1010	Leaf	Mo-26	
	Kondapur	18.175032	79.205462	Milky stage	MTU-1010	Neck	Mo-27	
	Kondapur	18.175032	79.205462	Milky stage	Devika gold	Leaf	Mo-28	
Medak	Ananthasagar	17.583264	78.292759	Mature grain	MTU1153	Leaf	Mo-29	
	Ambajipet	17.596072	78.232123	Mature grain	MTU1010	Leaf	Mo-30	

Agro climatic zone/	District	Place of collection	Latitude	Longitude	Stage of the crop	Variety	Host origin	Isolate code
Southern Telangana Zone	Rangareddy	IIRR, Rajendranagar	17.192100	78.234300	Nursery	HR 12	Leaf	Mo-31
		ARS, Rajendranagar	17.192100	78.234300	Tillering	BPT 5204	Leaf	Mo-32
	Vikarabad	Bandamedhapalli	18.819200	76.109700	Tillering	BPT 5204	Leaf	Mo-33
	Mahbubnagar	Noinapally	16.748294	77.995843	Tillering	RNR-15048	Leaf	Mo-34
		Hanwada	16.836397	77.904988	Tillering	Tellahamsa	Leaf	Mo-35
		Acharyapuram	16.749962	77.807755	Tillering	Tellahamsa	Leaf	Mo-36
	Jogulambagadwal	Rajoli	15.895930	77.828299	Mature grain	KNM 118	Leaf	Mo-37
		Shaikpally	16.127595	77.891530	Dough stage	MTU 1010	Leaf	Mo-38
	Suryapet	Alavalapuram	16.979427	80.010347	Tillering	HMT Sona	Leaf	Mo-39
		Aminabad	17.068332	79.965265	pancicle emergence	MTU 1010	Leaf	Mo-40

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## ROOT DYNAMICS OF AEROBIC RICE UNDER ORGANIC NUTRIENT SOURCES AND INORGANIC NUTRIENT LEVELS

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### ABSTRACT

A field experiment was conducted on root dynamics of aerobic rice under organic sources and inorganic nutrient levels during kharif, 2017 and 2018. The experiment was laid out in split plot design with four organic sources of nutrients as main plots viz: Neem leaf manure (@ 6 t ha<sup>-1</sup>), Vermicompost (@ 2 t ha<sup>-1</sup>), Goat manure (@ 5 t ha<sup>-1</sup>) and Microbial consortia (seed treatment @ 4 g kg<sup>-1</sup> + soil application @ 4 kg ha<sup>-1</sup>) and four subplots with graded doses of fertilizers viz: Control, 50 % RDF, 75 % RDF and 100 % RDF (120-60-40). The study revealed that various nutrient sources and inorganic nutrient levels exerted a remarkable effect on root traits viz: root length, root biomass, root volume and while root-shoot ratio was not impacted significantly by nutrient sources. Application of vermicompost (@ 2 t ha<sup>-1</sup>) or goat manure (@ 5 t ha<sup>-1</sup>) resulted in higher root length, root biomass and root volume. Inorganic nutrient levels of 100% RDF (120-60-40) produced higher root length, root biomass, root volume and root-shoot ratio than lower doses.

**Key words:** Aerobic rice, root traits, nutrient management

Rice (*Oryza sativa* L.) is the staple food crop of around half the world's population, grown over an area of 162.06 M ha globally with an annual production of 746.6 M t and productivity of 4661 kg ha<sup>-1</sup> (FAO, 2019-20). In Asia, the rice production is a key element for economic and social stability as more than two billion people depend on rice for their dietary requirements (Kadiyala, 2012). In India, rice occupies an area of 43.66 M ha with an average production of 118.87 M t and with productivity of 2423 kg ha<sup>-1</sup>. While in Telangana, rice area is 3.19 M ha with production of 11.12 M t and productivity of 3483 kg ha<sup>-1</sup> (DES, 2021).

Among the four rice ecosystems (viz., Irrigated Rice Eco System, Rainfed Upland Rice Eco System, rainfed Lowland Rice Eco System and Flood Prone Rice Eco System) irrigated rice dominates in both area and production. In terms of global rice productivity irrigated rice comprises of 55 and 75 per cent of area and rice production, respectively (Mahender *et al.*, 2015).

Tuong and Bouman (2005) estimated that by 2025, 15-20 million ha of irrigated rice is estimated to suffer with scarcity of water and calls for a major shift in rice cultivation system to aerobic rice system which not only improves the productivity but also provide economic security.

Aerobic rice is an alternative and contingent rice production system (Sreedevi *et al.*, 2014), wherein rice crop is cultivated under non-puddled and non-saturated soil conditions. This concept is mainly targeted for irrigated lowlands, less water available areas and uplands, with facilities for supplemental irrigation (Belder *et al.*, 2005). This system saves water input and increases water productivity by reducing water use during land preparation and limiting seepage, percolation and evaporation (Peng *et al.*, 2012).

Roots of plant play fundamental role in absorption of nutrients, water and stress tolerance affecting agricultural production. Fertilization can make



important changes in root traits. The response of root growth and development to its environment is an important aspect for understanding the aerobic adaptation (Bengough *et al.*, 2011). In case of higher value of root length, there is large probability to explore in to deeper layers and absorb more nutrients from the soil and this will help in increasing above ground biomass and increases resistance to different stresses (like drought conditions and low level of nutrients in the soil).

In aerobic system, the dominant form of nitrogen is nitrate and relatively little ammonia volatilization is expected after fertilizer nitrogen application. The alternate moist and dry soil conditions may stimulate nitrification-denitrification processes in dry sown rice, resulting in loss of nitrogen through  $N_2$  and  $N_2O$ . The differences in soil N dynamics and pathways of nitrogen losses in dry sown rice system may result in different fertilizer nitrogen recoveries. With even high nitrogen applications in aerobic rice, grain filling may be limited by a low contribution of post-anthesis assimilates (Zhang *et al.*, 2009). In addition, in the absence of transplanting, the roots of aerobic rice are located in the shallow surface soil, which results in a relatively low uptake of nitrogen (Zhang and Wang, 2002). Proper nutrient management not only ensures adequate supply of nutrients to plant but also minimizes losses and maximizes the nutrient use efficiency. Under such circumstances importance of organic manures and PGPR (Plant Growth Promoting Rhizo bacteria) is gaining prominence. Integrated nutrient management improves soil fertility besides sustaining the desired levels of crop production and productivity through optimization of benefits from all possible sources of plant nutrients (Kundu and Pillai, 1992). It entails the conjunctive use of compost, FYM, vermicompost, crop residues, green manures, crop rotation, bio fertilizers and inorganic fertilizers in a compatible manner to achieve sustainable yields. It not only reduces the dependence on chemical fertilizers but also improves the bio-physico-chemical properties. It encourages the growth and activity of mycorrhizae and other beneficial organisms in the soil, increases fertilizer use efficiency, alleviates the deficiency of secondary and micronutrients, sustains higher productivity and improved soil health (Singh *et al.*, 2006). Therefore, suitable combination of chemical fertilizers, organic manures and microbial cultures need to be developed that would result in

producing rice plants with better root traits in aerobic condition. Keeping this in view, an attempt was made to evaluate the performance of aerobic rice under organic nutrient sources and inorganic nutrient levels

## MATERIAL AND METHODS

Present field experiment was conducted during *kharif* in consecutive years, 2017 and 2018 at Research Farm, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. The farm is geographically situated at an altitude of 542.6 m above mean sea level (MASL) at 17° 19' N latitude and 78° 23' E longitude. The region is categorized under the Southern Telangana Agro-climatic zone under semi-arid tropic region (SAT).

The soil was sandy clay loam in texture with pH (1:2.5) of 8.14, Electrical conductivity (EC) 0.33 dS/m, low in organic C 4.1 g kg<sup>-1</sup> and available N (208 kg/ha). Soil was high in available P (28 kg P ha<sup>-1</sup>) and available K (382 kg K ha<sup>-1</sup>).

Experiment was laid out in split plot design comprising of four organic sources of nutrients as main treatments *viz*: M<sub>1</sub>: Neem leaf manure @ 6 t ha<sup>-1</sup>, M<sub>2</sub>: Vermicompost @ 2 t ha<sup>-1</sup>, M<sub>3</sub>: Goat manure @ 5 t ha<sup>-1</sup> and M<sub>4</sub>: Microbial consortia (seed treatment @ 4g kg<sup>-1</sup> + soil application @ 4 kg ha<sup>-1</sup>) and four fertilizer levels as sub treatments S<sub>1</sub>: Control, S<sub>2</sub>: 50% RDF, S<sub>3</sub>: 75 % RDF and S<sub>4</sub>: 100 % RDF, treatments were replicated thrice.

The field was dry-ploughed and harrowed but not puddled during land preparation. A seed rate of 30 kg ha<sup>-1</sup> was used. Seed was treated with carbendazim (1 g kg<sup>-1</sup>). The seed in the Microbial consortia treatment was treated with microbial consortia @ 4 g kg<sup>-1</sup>. Dry seed was sown at a inter-row spacing of 20 cm and intra-row spacing of 10 cm. Application of post emergence herbicide Bispyribacsodium @ 10 g *a.i.* ha<sup>-1</sup> was done at 12 DAS followed by one hand weeding at 45 DAS. Herbicide was sprayed using fluid volume of 500 l water ha<sup>-1</sup> with flood jet nozzle. Nitrogen, phosphorus and potassium fertilizer requirement of each of the individual treatment was determined and applied in the form of urea, single super phosphate and muriate of potash, respectively. Nitrogen dosage was applied in three equal splits *i.e.*,  $\frac{1}{3}$ <sup>rd</sup> as basal,  $\frac{1}{3}$ <sup>rd</sup> at active tillering and remaining  $\frac{1}{3}$ <sup>rd</sup> at panicle initiation stage. Entire

dose of  $P_2O_5$  was applied as basal and  $K_2O$  was applied in two equal splits  $\frac{1}{2}$  as basal  $\frac{1}{2}$  at panicle initiation stage. Iron deficiency was observed in aerobic rice at 20-30 DAS and Ferrous sulphate (@ 5 g l<sup>-1</sup>) was sprayed with knapsack sprayer twice at weekly interval.

Biometrical observations on root traits *viz*; root length, root dry weight, root volume and root-shoot ratio were determined at 15 days interval up to panicle initiation. The roots of five plants marked for destructive sampling were carefully removed with shovel, thoroughly washed and separated from shoot portion with knife. Root length was measured using scale from collar to tip of the longest root and expressed in cm. To measure root volume, root image analysis was accomplished by destructive method. The fresh root samples were properly washed with water and cleaned from any dirt/ decay materials. Before analysis, the roots were digitized with Epson Perfection V 700 Photo scanner. The analysis of scanned root was carried out by RHIZO-2012 software and volume of 5 roots was measured. the mean root volume is expressed as cc hill<sup>-1</sup>. For estimating root dry weight roots were dried in an oven at 70°C until constant weight was obtained and was expressed as g hill<sup>-1</sup>.

## RESULTS AND DISCUSSION

### Root length (cm)

Organic nutrient sources and graded fertilizer doses significantly influenced the root length (Table 1) of aerobic rice during *kharif* 2017 and 2018 at all growth intervals (30, 45, 60 and 75 DAS) except at 15 DAS. Root length had shown a progressive increase from 15 to 75 DAS and produced higher root length during *kharif* 2018 as compared to 2017.

The two-year pooled mean results of aerobic rice revealed that at 15 DAS the organic nutrient sources did not show significant effect on root length. However, from 30 to 75 DAS there was a note worthy variation in root length with applied organic manures. Vermi compost and goat manure produced significantly higher root length values of at 30, 45, 60 and 75 DAS than neem leaf manure and microbial consortia. Among the sub plots,  $S_4$  [100% RDF] had produced highest root length at all growth stages, while  $S_1$  [Control] had lowest root length 15 to 75 DAS. However, the interaction effect

between organic nutrient sources and on root length was found to be nonsignificant at all growth stages during both the years.

### Root biomass (g hill<sup>-1</sup>)

There was a progressive increase in root biomass from 15 to 75 DAS and higher root biomass was recorded during *kharif* 2018 compared to 2017 as presented in table 2. The two-year pooled mean results of aerobic rice revealed that, vermicompost and goat manure produced significantly higher root biomass at 30, 45, 60 and 75 DAS, over other two sources. Among sub plots,  $S_4$  [100% RDF] produced highest root biomass at all growth stages, while  $S_1$  [Control] recorded lowest root biomass at 15 to 75 DAS.

### Root volume (cc hill<sup>-1</sup>)

The response of root traits to applied nutrition is indicative of its ability to uptake more nutrient and water from the soil. The data pertaining to root volume as influenced by organic nutrient sources and graded fertilizer doses are given in Table 3. Two-year pooled mean analysis revealed that among different organic sources of nutrients,  $M_2$  [vermicompost @ 2 t ha<sup>-1</sup>] registered highest root volume but was statistically at par with  $M_3$  [goat manure @ 5 t ha<sup>-1</sup>]. Both vermicompost and goat manure recorded significantly higher root volume than neem leaf manure and microbial consortium. The increase in nutrient dose resulted in significant enhancement in root volume and highest root volume was recorded with application of highest dose of nutrients 100% RDF  $\{S_4\}$  followed by 75% RDF  $\{S_3\}$  50% RDF  $\{S_2\}$  and least root volume were put forth by Control  $\{S_1\}$ .

However, the interaction effect of organic sources nutrients and inorganic nutrient level was found to be non-significant on root volume.

### Root-shoot ratio

Root-shoot ratio decreased progressively with advancement of age 15, 30, 45, 60 and 75 DAS. There was no difference with the organic nutrient source applied on Root -Shoot ratio at all growth stages during both the years of study. With increase in fertilizer level there was a significant enhancement in root-shoot ratio  $S_4$  [100% RDF] produced highest root-shoot ratio while  $S_1$  [Control] had lowest root: shoot ratio of from 15 to 75 DAS.

ROOT DYNAMICS OF AEROBIC RICE

Table 1. Root length (cm) of aerobic rice as influenced by organic nutrient sources and inorganic nutrient levels during *kharif*, 2017 and 2018.

Treatment	15 DAS	30DAS	45 DAS	60 DAS	75 DAS
<b>Organic nutrient sources (M)</b>					
M <sub>1</sub> : Neem leaf manure 6 t ha <sup>-1</sup>	7.90	10.11	12.21	15.10	18.09
M <sub>2</sub> : Vermicompost 2t ha <sup>-1</sup>	8.76	12.51	16.34	20.64	24.43
M <sub>3</sub> : Goat manure 5 t ha <sup>-1</sup>	7.39	9.85	15.33	19.02	22.65
M <sub>4</sub> : Microbial consortia ST 4g /kg & SA 4kg ha <sup>-1</sup>	8.26	11.85	15.28	17.23	19.07
SEm±	0.21	0.32	0.42	0.53	0.63
CD (P=0.05)	NS	1.10	1.45	1.84	2.16
<b>Inorganic nutrient levels (S)</b>					
S <sub>1</sub> : 0% RDF	4.84	6.47	8.44	10.86	12.81
S <sub>2</sub> : 50%RDF	7.19	9.49	12.39	15.27	18.47
S <sub>3</sub> : 75%RDF	9.45	13.01	16.97	20.93	24.74
S <sub>4</sub> : 100%RDF	10.82	14.87	19.39	23.92	28.21
SEm±	0.18	0.40	0.27	0.33	0.39
CD (P=0.05)	0.53	0.40	0.78	0.97	1.15
<b>Interaction</b>					
<b>M X S</b>					
SEm±	7.90	7.90	7.90	7.90	7.90
CD (P=0.05)	NS	NS	NS	NS	NS
<b>S X M</b>					
SEm±	0.52	0.59	0.67	0.72	0.82
CD (P=0.05)	NS	NS	NS	NS	NS

Note: :ST: Seed Treatment, SA: Soil Application & RDF: Recommended dose of fertilizer :120:60:40 NPK

Table 2. Root biomass (g hill<sup>-1</sup>) of aerobic rice as influenced by organic nutrient sources and inorganic nutrient levels during kharif 2017 and 2018

Treatment	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
<b>Organic nutrient sources (M)</b>					
M <sub>1</sub> : Neem leaf manure 6 t ha <sup>-1</sup>	2.17	3.59	5.16	7.46	9.45
M <sub>2</sub> : Vermicompost 2t ha <sup>-1</sup>	2.66	4.50	7.04	9.66	11.57
M <sub>3</sub> : Goat manure 5 t ha <sup>-1</sup>	1.82	3.10	6.73	9.18	11.02
M <sub>4</sub> : Microbial consortia ST 4g /kg & SA 4kg ha <sup>-1</sup>	2.42	4.13	5.51	7.96	9.74
SEM±	0.09	0.14	0.10	0.14	0.17
CD (P=0.05)	NS	0.50	0.35	0.49	0.58
<b>Inorganic nutrient levels (S)</b>					
S <sub>1</sub> : 0% RDF	1.82	2.65	3.48	5.20	6.66
S <sub>2</sub> : 50%RDF	2.02	3.46	5.36	7.76	9.68
S <sub>3</sub> : 75%RDF	2.33	4.08	7.06	9.75	11.84
S <sub>4</sub> : 100%RDF	2.90	5.12	8.54	11.56	13.60
SEM±	0.13	0.20	0.14	0.20	0.25
CD (P=0.05)	0.37	0.43	0.42	0.59	0.73
<b>Interaction</b>					
<b>M X S</b>					
SEM±	0.25	0.43	0.29	0.41	0.25
CD (P=0.05)	NS	NS	NS	NS	NS
<b>S X M</b>					
SEM±	0.19	0.31	0.21	0.30	0.37
CD (P=0.05)	NS	NS	NS	NS	NS

Note: ST: Seed Treatment, SA: Soil Application & RDF: Recommended dose of fertilizer :120:60:40 NPK

Table 3. Root volume (cc hill<sup>-1</sup>) of aerobic rice as influenced by organic nutrient sources and inorganic nutrient levels during *Kharif 2017 and 2008*

Treatment	15 DAS	30DAS	45 DAS	60 DAS	75 DAS
<b>Organic nutrient sources (M)</b>					
M <sub>1</sub> : Neem leaf manure 6 t ha <sup>-1</sup>	6.98	10.78	13.12	16.75	20.98
M <sub>2</sub> : Vermicompost 2t ha <sup>-1</sup>	8.03	12.21	16.62	21.39	25.49
M <sub>3</sub> : Goat manure 5 t ha <sup>-1</sup>	6.20	10.24	14.89	20.06	24.47
M <sub>4</sub> : Microbial consortia ST 4g /kg & SA 4kg ha <sup>-1</sup>	7.46	11.61	14.55	18.09	22.74
SEm±	0.34	0.24	0.25	0.47	0.57
CD (P=0.05)	NS	0.56	0.88	1.62	1.96
<b>Inorganic nutrient levels (S)</b>					
S <sub>1</sub> : 0% RDF	4.99	7.90	10.48	12.65	17.18
S <sub>2</sub> : 50%RDF	5.94	10.37	14.50	17.89	22.52
S <sub>3</sub> : 75%RDF	7.81	11.94	16.27	21.06	25.40
S <sub>4</sub> : 100%RDF	9.93	14.51	18.93	24.69	28.58
SEm±	0.39	0.29	0.40	0.47	0.50
CD (P=0.05)	1.13	0.86	1.16	1.36	1.47
<b>Interaction</b>					
<b>M X S</b>					
SEm±	0.77	0.59	0.79	0.93	1.01
CD (P=0.05)	NS	NS	NS	NS	NS
<b>S X M</b>					
SEm±	0.63	0.47	0.58	0.81	0.93
CD (P=0.05)	NS	NS	NS	NS	NS

Table 4. Root-shoot ratio of aerobic rice as influenced by organic nutrient sources and inorganic nutrient levels during *kharrif*, 2017 & 2018.

Treatment	15 DAS	30DAS	45 DAS	60 DAS	75 DAS
<b>Organic nutrient sources (M)</b>					
M <sub>1</sub> : Neem leaf manure 6 t ha <sup>-1</sup>	0.53	0.42	0.34	0.30	0.27
M <sub>2</sub> : Vermicompost 2t ha <sup>-1</sup>	0.56	0.45	0.38	0.34	0.33
M <sub>3</sub> : Goat manure 5 t ha <sup>-1</sup>	0.55	0.44	0.37	0.33	0.31
M <sub>4</sub> : Microbial consortia ST 4g /kg & SA 4kg ha <sup>-1</sup>	0.54	0.43	0.36	0.32	0.29
SEm±	0.011	0.009	0.006	0.006	0.007
CD (P=0.05)	NS	NS	NS	NS	NS
<b>Inorganic nutrient levels (S)</b>					
S <sub>1</sub> : 0% RDF	0.39	0.37	0.28	0.24	0.22
S <sub>2</sub> : 50% RDF	0.49	0.42	0.34	0.30	0.27
S <sub>3</sub> : 75% RDF	0.58	0.46	0.39	0.35	0.33
S <sub>4</sub> : 100% RDF	0.73	0.51	0.45	0.41	0.39
SEm±	0.008	0.006	0.005	0.004	0.006
CD (P=0.05)	NS	0.018	0.014	0.012	0.017
<b>Interaction</b>					
<b>M X S</b>					
SEm±	0.015	0.013	0.009	0.008	0.012
CD (P=0.05)	NS	NS	NS	NS	NS
<b>S X M</b>					
SEm±	0.017	0.013	0.010	0.009	0.011
CD (P=0.05)	NS	NS	NS	NS	NS

Note: ST: Seed Treatment, SA: Soil Application &amp; RDF: Recommended dose of fertilizer :120:60:40 NPK

## ROOT DYNAMICS OF AEROBIC RICE

Interaction effect between organic nutrient sources and inorganic nutrient level on root-shoot ratio of aerobic rice was found to be non-significant at all growth stages during both the years (Table 4).

### CONCLUSION

The positive effect of organic manures vermicompost and goat manure on root traits viz; root length, root biomass, root volume and root-shoot ratio can be attributed to the enhancement soil fertility due

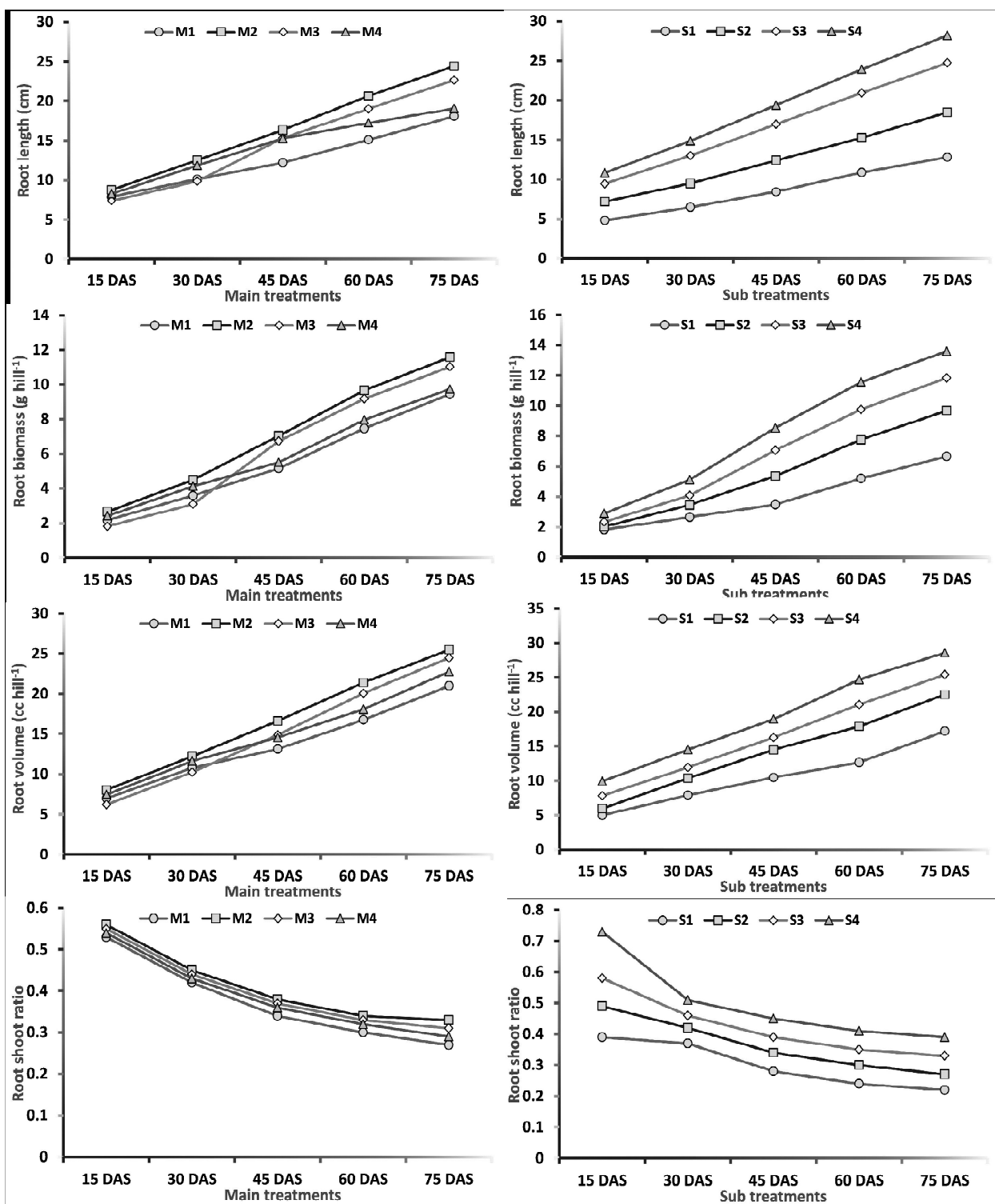


Figure 1. Aerobic rice root traits as influenced by organic nutrient sources and inorganic nutrient levels

to the application. They improve soil physical properties and make available plant nutrients and other growth promoting substances during different growth stages, resulting in improved root penetration, nutrients and moisture absorption, thus accelerating meristematic cellular activity, expressing morphologically in terms of higher root growth traits as corroborated by Rajanna (2012), Siddaram *et al.*, (2009), Supreet *et al.*, (2018) and Jana *et al.*, (2020).

Enhanced level of nutrient application in balanced proportion stimulated the elongation of adventitious roots and increased the root length, root volume. (Dong *et al.*, 2001), root biomass production and higher root-shoot ratio (Maheshwari *et al.*, 2007; Patil *et al.*, 2013; Anil *et al.*, 2018 and Singh *et al.*, 2020).

The interaction effect of different organic nutrient sources and fertilizer levels was non-significant during both the years of study.

From the present study it can be concluded that the organic sources of nutrients and graded doses of fertilizer have put forth a significant impact on root traits of aerobic rice. Application of vermicompost @ 2t ha<sup>-1</sup> or goat manure @ 5t ha<sup>-1</sup> among the nutrient sources and recommended dose of 120-60-40 kg ha<sup>-1</sup> should be applied for aerobic rice under SAT.

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## GENETIC ANALYSIS AND TRAITS ASSOCIATION IN RICE (*Oryza sativa* L.) UNDER SALT-STRESS CONDITIONS

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### ABSTRACT

Salinity stress is one of the main problems for the rice crop, as it reduces the production and productivity of the grain yield significantly. Genetic variability studies provide basic information concerning the genetic properties of the population based on which breeding methods could be formulated for further improvement of the crop. The estimates of heritability, coefficients of variability and genetic advance was computed in F<sub>2</sub>s of 24 crosses for 10 characters including yield, yield contributing traits and salt-tolerance under salinity condition during Kharif 2021. A joint consideration of PCV, GCV, heritability broad sense and genetic advance as percentage revealed that spikelet sterility percentage (62.01, 52.35, 71.3 and 18.14%), number of unfilled grains per panicle (50.42, 39.23, 60.5 and 25.54%) and total number of grains per panicle (26.75, 25.25, 89.1 and 109.76%) combined high PCV, GCV, high broad sense heritability and high genetic advance. High heritability coupled with high and moderate genetic advance was observed for all the plant traits under study except for days to 50% flowering and panicle length. Correlation studies revealed that seed yield per plant was positively and significantly correlated with number of productive tillers per plant and total number of filled grains per panicle. However seed yield plant per plant was negatively significantly associated with sterility percentage.

**Keywords:** Rice; Salinity; Genetic variability; Heritability; Genetic advance; Correlation; Path analysis

Rice is the most important staple food crop of the world and major source of calories for more than half of the global population. Rice, being the staple food for more than 70 per cent of our national population and source of livelihood for 120-150 million rural households, is backbone to the Indian Agriculture. Thus, the development of improved high yielding pure line and hybrid rice varieties suitable for adverse condition (salt affected soil) would be one of the important strategy to meet this challenge in context of production and productivity in salt affected soils.

Globally, rice is impacted by various abiotic stresses, with salinity being the second most severe abiotic stress after drought in terms of interfering with rice production and productivity. Electrical conductivity (EC) greater than 4 dSm<sup>-1</sup>, exchangeable sodium percentage (ESP) less than 15, and pH less than 8.5 are the indicators of salinity. Rice, being a glycophyte, is sensitive to salinity and has a strong sensitivity to the negative effects of salt accumulation.

The main effect of salinity is decrease in crop yield and production as a consequence of enhanced salt accumulation.

The knowledge of factors responsible for high yields has been rendered difficult since yield is a complex character that manifests through multiplicative interactions of other characters known as yield components (Grafius, 1959). Therefore, the identification of important yield contributing characters, out of numerous plant traits, is necessary because it would be impossible and impractical to concentrate and work on improving many characters at a time. Moreover, before launching any breeding programme, a breeder should have a thorough knowledge on nature and magnitude of genetic variability, heritability and genetic advance in a crop species.

The correlation and path coefficient analysis help us in identification of important yield contributing characters. The coefficient of correlation expresses association between two variables, but tells us nothing about the causal relations of variables, i.e.,

which variable is dependent and which is independent. Therefore, the study of path-coefficients is necessary. Path-coefficient is simply a standardized partial regression coefficient, which splits the correlation coefficient into the measures of direct and indirect effects. It also estimates residual effects. Path analysis clearly indicates the relative importance of different yield components so that one may identify the most important yield components. Since yield is inherited in a complex way and is influenced by the environment, path coefficient analysis will be an added advantage to the breeder in crop improvement programme.

Salt affected areas have increased day by day because of excessive use of irrigation water with improper drainage coupled with poor quality irrigation water. The salt tolerant rice varieties are sparse and for the development of high yielding pure line and hybrid varieties in rice, the information on various genetic aspects in respect to important plant characters is essential for planning and execution of a successful breeding programme. The understanding of genetic architecture and direct and indirect selection parameters of agronomically important traits helps in deciding the type of variety to be developed and the breeding methodology to be followed in a particular growing situation. In order to develop high yield pure line rice varieties, it is essential to screen genotypes for variability, heritability, correlation and path analysis for different characters which is prerequisite for identification of potential rice varieties for the adverse soil conditions. Although, the information on the above aspects in rice is available, but most of these studies are based on salt-stress conditions and literature based on salinity conditions are meagre.

## **MATERIAL AND METHODS**

### **Crossing and development of F<sub>1</sub> population**

Twenty-four crosses were generated using L X T mating design by crossing six female lines viz., KPS 10628, KPS 10631, KPS 10633, KPS 10640, KPS 10642 and KPS 10651 with four testers CSR 23, CSR 36, RNR 11718 and KPS 2874. Three staggered sowing of the parental genotypes was done to achieve synchronization for effective crossing programme to generate F<sub>1</sub>. The seedlings were raised during Rabi 2020-2021 following all the

recommended agronomic practices. At flowering stage, the florets of female parents were hand emasculated early in the morning, before 7 a.m. and later the pollen was collected from male parent and dusted on to the stigma around 10 a.m which is the ideal time for effective pollination. To avoid contaminations from foreign pollens, emasculated panicles were covered with butter paper packet. The seeds set on female plants were harvested, around 25-27 days after crossing event. Twenty four crosses were effected in a pair wise combination during Rabi 2020-2021, at ARS, Kampsagar.

### **Evaluation of F<sub>1</sub> generation population**

Seedlings that had been growing for 25 days were transplanted into the main field with a 20 x 15 cm spacing during *Kharif* 2021 with maintaining E.C. 4ds/m and p<sup>H</sup> 9.2. 24 F<sub>1</sub> hybrids, their parents, and three high yielding check varieties, were grown in a randomized complete blocks design (RCBD) with three replications. The study's experimental material was cultivated using a suggested set of techniques for raising a healthy crop. To generate a good harvest, necessary plant protection measures were employed at the appropriate time. Ten components for yield and yield attributing features were observed, where as data on days to 50% flowering and seedling mortality were collected on plot basis, while other traits were collected on five randomly selected plants. Mean values were utilized for statistical analysis and the characters observed for eliciting the information are: Mortality percentage, days to 50 percent flowering, plant height (cm), panicle length (cm), number of productive tillers/m<sup>2</sup>, Total number of grains per panicle, Number of unfilled grains per panicle, spikelet sterility(%), 1000 grain weight (g), and Seed yield per plant (g) were recorded.

### **STATISTICAL ANALYSIS**

The observations recorded in respect of all the above quantitative traits were subjected to following standard statistical analysis. The mean values of quantitative traits were used to estimate genetic variability parameters, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h<sup>2</sup>), genetic advance (GA), correlation and path analysis. Statistical analysis was done through WINDOSTAT version 9.2 software.

## RESULTS AND DISCUSSION

### Genetic Variability

Genotypic and phenotypic coefficient of variation, heritability, genetic advance and genetic advance as percentage of mean were estimated for yield and yield components in  $F_1$  generation as presented in Table 1. Expectedly phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) in all the characters studied. The difference between PCV and GCV is probably due to environmental effects.

High heritability estimates for all the characters except panicle length and 1000 grain weight suggesting that the environmental factors did not affect greatly the phenotypic performance of these traits. Highest PCV (62.01 %) and GCV (52.35 %) were observed for spikelet sterility percentage. PCV generally ranged between 6.17 % for 1000 grain weight to 62.01 % for spikelet sterility percentage indicating the presence of inherent variability among the lines. Similarly, GCV ranged between 1.63 % for 1000 grain weight to 52.35 % for spikelet sterility percentage. A similar finding of higher PCV than GCV was also reported by Parimala and Devi (2019), and Hasan-Ud-Daula and Sarker (2020).

Moderate estimates of GCV and PCV were observed in the case of plant height (cm), panicle length (cm) and seed yield per plant (g). These findings were in line with the findings of Sumanth *et al.*, (2017) for seed yield per plant. Low estimates of GCV and PCV were observed in the case of days to 50 % flowering and 1000 grain weight indicating a lack of inherent variability and limited scope of improvement through selection for these traits among the genotypes. Similar results were observed by Singh and Varma (2018).

According to Johnson, broad sense heritability is classified as low (<30%), moderate (30-60%) and high (>60%). This shows most of the traits studied can be easily improved through selection except for 1000 grain weight (g) and panicle length (cm). Generally, heritability in broad sense estimate varied from 7.10 % for 1000 grain weight, 14.7 % for panicle length and 98.1% for mortality percentage, respectively. The high heritability values of the considered traits in the present study indicated that these are less influenced by the

environment and selection of the traits can be done based on the phenotypic expression by adopting a simple selection method. A similar case of low estimate was reported by Swapnil *et al.*, (2020) for panicle length, whereas Devi *et al.*, (2017), Saha *et al.*, (2019), and Hasan-Ud-Daula and Sarker (2020) reported high estimates for remaining traits.

Genetic advance was ranged between 0.20 % for 1000 grain weight and 251.44 % for number of productive tillers/m<sup>2</sup>. Genetic advance was low for days to 50 % flowering, panicle length, seed yield/plant and 1000 grain weight (g). Moderate values were observed in the case of traits such as mortality (%), plant height (cm), and sterility percentage. A high value of genetic advance was observed in the case of number of productive tillers/m<sup>2</sup>, total number of filled grains/panicle and unfilled grains/panicle. A similar case of the low estimate for days to 50 % flowering was reported by Hasan-Ud-Daula and Sarker (2020) whereas Divya *et al.*, (2018), and Singh and Varma (2018) reported for panicle length, Manohara and Singh (2015) for the number of filled grains per panicle and Parimala and Devi (2019) for seed yield/plant.

Genetic advance as percent of mean was low for days to 50 % flowering, panicle length and 1000 grain weight. Moderate values were found in the case of plant height. High values were found in the case of mortality (%), number of productive tillers/m<sup>2</sup>, total number of grain/panicle, number of unfilled grains/panicle, sterility percentage (%) and seed yield/plant. These findings align with the reports of Sumanth *et al.*, (2017) for days to 50 % flowering, Islam *et al.*, (2016) in case of panicle length (cm), Divya *et al.* (2018) for plant height (cm), Saha *et al.*, (2019), and Sameera *et al.*, (2016) for number of productive tillers/hill number of grains/panicle, number of filled grains/panicle. Hasan-Ud-Daula and Sarker (2020) for sterility percentage (%), Devi *et al.*, (2017) for seed yield/plant.

High estimates of heritability and genetic advance were observed in the case of traits such as mortality (%), number of productive tillers/m<sup>2</sup>, total number of grains per panicle, number of unfilled grains/panicle, sterility percentage (%), and seed yield/plant (g). It indicates the role of additive gene action and would facilitate better scope for improve-

ment of these traits through direct positive selection, but mortality (%) and sterility percentage (%) are of negative importance to yield, so negative selection should be applied for these traits. Thus, high estimates of GCV and heritability could be good predictors of seed yield in rice, and selection based on the phenotypic performance will be reliable and effective.

Furthermore, moderate to high heritability, GCV and GA% in mean could be explained by additive gene action and their improvement could be achieved through mass selection (Table 1). In the case of traits with high heritability with low or moderate genetic advance, indicates the influence of environment over the character facilitating the possibility of improvement by intermitting superior genotypes of segregating populations developed from combination breeding (Rasel *et al.*, 2018).

### Correlation

Yield is a complex trait being influenced by several interdependent quantitative characters. Selection for yield may not be effective unless other yield components influencing it directly or indirectly are taken into consideration. When selection pressure is

exercised for the improving of any trait highly associated with yield, simultaneously it affects many other correlated traits. Hence, knowledge regarding the correspondence of character with yield and among themselves provides a guideline to the breeder for improving through selection contribution in respect of establishing the association by genetic and non-genetic factors (Table 2).

### Correlation of seed yield with different traits:

The grain yield/plant exhibited a highly significant and positive correlation with number of productive tillers/m<sup>2</sup> (0.71\*\*), total number of filled grains/panicle (0.57\*\*). Positive correlation was also recorded for panicle length (0.28) and 1000 grain weight (0.20) which was also observed by UI Islam *et al.* (2017), Saha *et al.* (2019) and Shrivatsav *et al.* (2020). On the other hand, significant negative correlation was recorded for days to 50% flowering (-0.40\*), unfilled grains/panicle (-0.41\*) and sterility percentage (-0.64\*\*), mortality percentage (-0.23) and plant height (-0.24), which was also observed by Touhiduzzaman *et al.* (2016). Highly significant negative correlation with

**Table 1. Estimates of PCV, GCV, heritability (broad sense) GA% and GA% mean in F1 generation**

S. No.	Trait	Mean	Range		PCV (%)	GCV (%)	h <sup>2</sup>	GAP	GAM
			L	H					
1.	M (%)	21.8	6.1	32.2	40.6	40.2	98.1	17.9	82.1
2.	DFF	94.7	83.6	113.0	6.6	5.4	66.8	8.7	9.2
3.	PH(cm)	91.0	74.2	111.2	11.2	10.2	82.3	17.3	19.0
4.	PL(cm)	23.3	20.4	27.8	11.7	4.5	14.7	0.8	3.5
5.	NPT/m <sup>2</sup>	480.8	202.9	790.9	32.8	28.8	77.4	251.4	52.3
6.	TNGP	223.6	149.6	351.3	26.7	25.2	89.1	109.7	49.0
7.	UFG	40.6	9.6	78.0	50.4	39.2	60.5	25.5	62.8
8.	S%	19.9	4.3	48.8	62.0	52.3	71.3	18.1	91.0
9.	TW (g)	22.3	21.0	24.5	6.1	1.6	7.1	0.2	8.9
10.	SY (g)	26.2	17.1	32.0	18.0	15.5	74.9	7.2	27.8

PCV= Phenotypic Coefficient of variation  
h<sup>2</sup>= Heritability  
GAM= Genetic advance as a percent of the mean (at 5%)  
L-Lower mean  
MT (%) Mortality  
PH (cm) - Plant height  
NPT- No. of Productive tillers/m<sup>2</sup>  
UFG - No. of unfilled grains/panicle  
TW (g) - Test weight

GCV= Genotypic Coefficient of variation  
GAP= Genetic advance percent (at 5%)

H-Higher mean  
DFF- Days to 50% flowering  
PL (cm) - Panicle length  
TNGP - Total no. of grains/panicle  
SP (%) - Sterility %  
SY (g) - Seed yield per plant

**Table 2. Phenotypic correlations for the yield and yield contributing traits in F<sub>1</sub> generation.**

	MT (%)	DFF	PH	PL	NPT/m <sup>2</sup>	TNGP	UFG	SP (%)	TW	SY
MT (%)	1.00	0.09	-0.06	0.18	-0.51 **	-0.54 **	0.01	0.17	-0.17	-0.23
DFF		1.00	0.23	0.19	-0.17	-0.22	0.17	0.24	-0.41 **	-0.40 *
PH			1.00	-0.16	-0.03	-0.02	0.16	0.16	0.20	-0.24
PL				1.00	0.16	0.03	-0.47 **	-0.38 *	0.07	0.28
NPT/m <sup>2</sup>					1.00	0.80 **	-0.38 *	-0.66 **	0.10	0.71 **
TNGP						1.00	-0.23	-0.58 **	0.22	0.57 **
UFG							1.00	0.90 **	0.01	-0.41 *
SP (%)								1.00	-0.06	-0.64 **
TW									1.00	0.20
SY										1.00

\* - significant at 5 per cent level

\*\* - significant at 1 per cent level

MT (%) - Mortality

DFF - Days to 50 % flowering

PH (cm) - Plant height

PL (cm) - Panicle length

NPT - No. of Productive tillers/m<sup>2</sup>

TNGP - Total no. of grains/panicle

UFG - No. of unfilled grains/panicle

SP (%) - Sterility %

TGW (g) - Test weight

SY (g) - Seed yield/plant

sterility percentage was observed by Narayanan *et al.*, (2019) and Ghazy *et al.*, (2020).

Due to increase in tiller number, the number of filled grains will increase and thereby increase in seed yield. Due to increase of salt stress conditions, plants will change biological system *viz.*, early flowering and reduced height, the seed yield was increased due to reduced plant height, which are negatively correlated.

#### Correlation among different traits:

Mortality percentage was significant and negatively correlated with number of productive tillers/m<sup>2</sup> (-0.51\*\*) and total number of grains/panicle (-0.54\*\*). With decrease in mortality percent, tiller count and grain number has increased and resulted in increase of seed yield (Sarawgi *et al.*, 1997). Number of productive tillers/m<sup>2</sup> was highly positive correlated with total number of filled grains/panicle (0.80\*\*) and highly negatively correlated with number of unfilled grains/panicle (-0.38\*) and sterility percentage (-0.66\*\*). Similar results was reported by Vennila and Palaniraja (2018) and Aarthi *et al.* (2019). Unfilled grains/ panicle is significant and positive association

with sterility percentage (0.90\*\*) (Touhiduzzaman *et al.*, 2016). Sterility percentage is highly negative significant association with number of productive tillers/plant and total number of filled grains/panicle (Sarawgi *et al.*, 1997). Positive non significant association was observed with characters *i.e.*, mortality percentage with days to 50% flowering, plant height with days to 50% flowering, panicle length with number of productive tillers/plant and total number of grains/panicle with panicle length. Similar results was reported by Touhiduzzaman *et al.* (2016), Gautam *et al.* (2018) and Narayanan *et al.* (2019). Panicle length is negative significantly associated with unfilled grains/panicle and sterility percentage, and due to stress conditions panicle length was increased and results in less number of unfilled grains, therefore decreases the sterility percentage.

#### Path Analysis

The path analysis is a useful parameter to understand more clearly the association among different variables as recorded by simple correlation values. It helps to partition the overall association of

particular variables with dependent variable into direct and indirect effects. While dealing with a more complex character like seed yield, it enables the breeder to specifically identify the important component trait of such a nature and differential emphasis can be laid on those characters for selection. Path coefficient analysis of yield and its component traits (Table 3) revealed that number of unfilled grains/panicle (1.20) had the highest positive direct effect on seed yield. The number of productive tillers/m<sup>2</sup> (0.35) and panicle length (0.13) was the second most important characters as they showed highest positive direct effect on seed yield, and therefore indirect selection based on number of productive tillers/m<sup>2</sup> and more panicle length can be adopted for enhancement of seed yield. Sterility percentage (-1.61) had showed highest negative direct effect on seed yield.

The mortality percentage, days to 50% flowering, plant height, total number of grains /panicle and sterility percentage has negative direct on seed yield. Similar results was reported by Patel *et al.* (2014), Singh *et al.* (2016), Prasad *et al.* (2017), Tripathi *et al.* (2018), Shrivatsav *et al.* (2020) and Hasan-Ud-Daula

and Sarker (2020). The number of productive tillers/plant had negative indirect effect on grain yield via mortality percentage, days to 50% flowering, plant height, number of unfilled grains/panicle, and sterility percentage. Similar results were also reported by Karthikeyan *et al.* (2019), Saha *et al.* (2019), Sumithra *et al.* (2019) and Kiruthikadevi *et al.* (2020).

The traits that have shown positive direct effect on seed yield helps in selection of that trait directly to enhance the yield. The high residual effect indicated that different characters other than the characters considered in this study also influences the grain yield considerably (Table 3 and Figure 1).

Mortality percentage had negative indirect effect on grain yield via days to 50% flowering, panicle length, sterility percentage and 1000 grain weight. Sterility percentage has negative indirect effect with grain yield via mortality percentage, days to 50% flowering, panicle length, plant height, number of productive tillers per plant and 1000 grain weight. A similar case was reported by Sarawgi *et al.* (1997), Karim *et al.* (2014) and Saha *et al.* (2019).

**Table 3. Phenotypic direct (diagonal) and indirect effects of different quantitative traits in F<sub>1</sub> generation.**

	MT (%)	DFF	PH	NPT/m <sup>2</sup>	PL	TNGP	UFG	SP (%)	TW
MT (%)	<b>-0.032</b>	-0.003	0.002	0.015	-0.004	0.016	-0.000	-0.005	0.003
DFF	-0.017	<b>-0.187</b>	-0.039	0.023	-0.019	0.033	-0.017	-0.029	0.036
PH	0.006	-0.024	<b>-0.114</b>	0.003	0.010	0.003	-0.013	-0.014	-0.011
NPT/m <sup>2</sup>	-0.168	-0.043	-0.011	<b>0.354</b>	0.025	0.248	-0.124	-0.205	-0.008
PL	0.019	0.014	-0.012	0.010	<b>0.139</b>	0.008	-0.041	-0.036	0.014
TNGP	0.232	0.080	0.012	-0.317	-0.028	<b>-0.452</b>	0.100	0.248	-0.054
UFG	0.018	0.114	0.138	-0.424	-0.362	-0.266	<b>1.208</b>	1.101	-0.036
SP (%)	-0.261	-0.257	-0.199	0.940	0.423	0.887	-1.475	<b>-1.619</b>	0.103
TW	-0.009	-0.019	0.010	-0.002	0.010	0.012	-0.003	-0.006	<b>0.102</b>
SY	-0.213	-0.326	-0.213	0.602	0.194	0.490	-0.367	-0.567	0.148

**Residual Effect - 0.6322**

MT (%)	- Mortality	DFF	- Days to 50 % flowering
PH (cm)	- Plant height	PL (cm)	- Panicle length
NPT	- No. of Productive tillers/m <sup>2</sup>	TNGP	- Total no. of grains/panicle
UFG	- No. of unfilled grains/panicle	SP (%)	- Sterility %
TW (g)	- 1000 grain weight	SY (g)	- Seed yield/plant

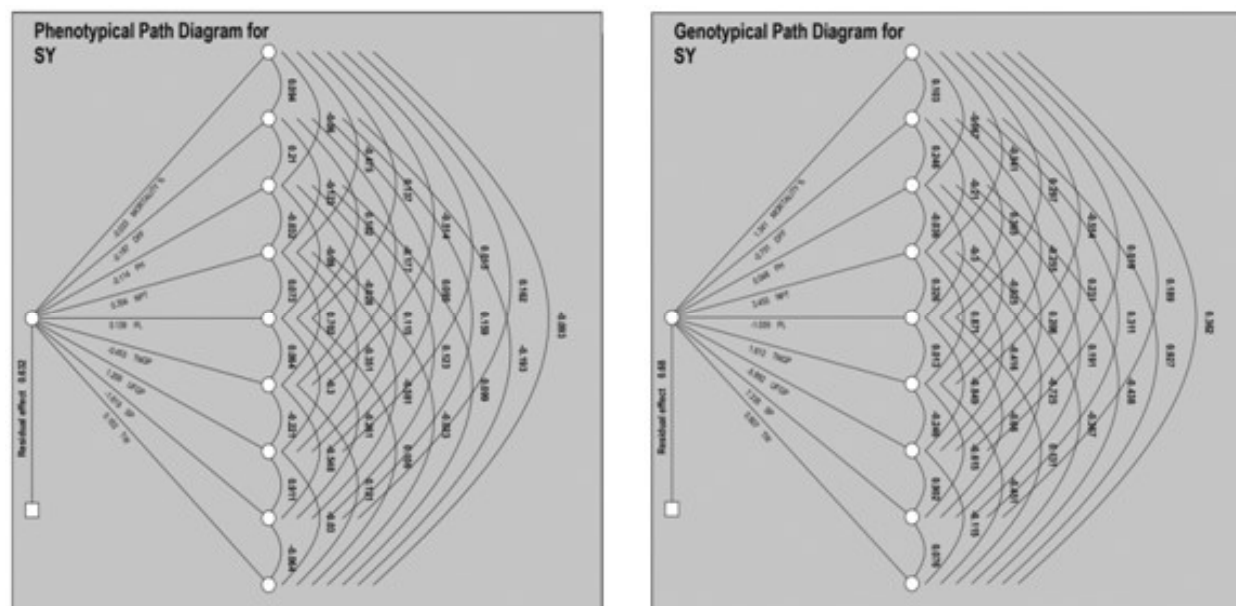


Figure 1. Phenotypical and genotypical path diagram in rice.

### Residual Effect

The high phenotypical residual value (0.632) and the high genotypical residue effect (0.680), is due to the influence of other traits. The association of different component characters among themselves and with yield is quite important for devising an efficient selection criterion for yield. Hence, indirect selection by correlated response may not be sometimes fruitful, when many characters are affecting a given character, splitting the total correlation into direct and indirect effects of cause as devised by Wright (1921) would give more meaningful interpretation to the cause of the association between the dependent variable like yield and independent variables like yield components. This kind of information will help formulate the selection criteria, indicate the selection for these characters is likely to bring about an overall improvement in single plant yield directly. Path analysis revealed that selection for the trait number of productive tillers/m<sup>2</sup> and total number of grains/panicle have to be given prior importance during selection, since they had a positive correlation along with positive direct effect on yield.

### CONCLUSION

An overall consideration of the results revealed that seed yield/plant could be improved through selection for traits like number of productive tillers/m<sup>2</sup>

and total number of grains/panicle, which were positively correlated and shown positive direct effect with seed yield/plant. Hence, it may be declared that selection and manipulation of number of productive tillers/m<sup>2</sup> and total number of grains/panicle would simultaneously improve seed yield/plant. High heritability estimates for all the characters except for panicle length revealed that selection for these characters will have to be carried in advance segregating generations.

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## IMPACT OF CHEMICAL MUTAGENESIS USING ETHYL METHANE SULPHONATE ON CASTOR SEED GERMINATION AND SEEDLING VIGOUR

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### ABSTRACT

Mutations are the primary source of all genetic variations existing in any organism, including plants. EMS-induced mutation is a highly successful strategy for developing superior crop varieties and it is widely employed in crop breeding. To the optimum conditions for mutagen treatment, the seeds of castor genotype DCH-519 was soaked in water for 24 h. The pre-soaked seeds were treated with the chemical mutagen, Ethyl Methane Sulphonate (EMS) with four different concentrations (0.25%, 0.5%, 1.0%, 1.5%) at four different time durations (4, 8, 12, 16 h). The effect of treatment on germination, seedling length, seedling vigour index - I (SV-I) and seedling vigour index - II (SV-II) was studied. It was observed that the germination percentage, speed of germination, seedling length and seedling vigour index decreases with increasing concentrations of EMS. The LD<sub>50</sub> (Lethal dose) value was determined based upon the seed germination percentage.

**Key words:** Castor, Chemical mutagenesis, EMS, Germination, Seedling vigour

Castor (*Ricinus communis* L.) is a member of the spurge family (*Euphorbiaceae*). It is a native plant of Ethiopia and North Africa. (Ramanjaneyulu *et al.*, 2017) It is one of the most important oilseed crops of arid and semi-arid regions with immense industrial and medicinal values. India, China, Brazil, Mozambique, Ethiopia and Thailand are the leading producers of castor contributing to 95 per cent of world production (<http://www.fao.org/faostat/>). India produces about 73 per cent of the world's castor (FAO, 2018) with an annual production of roughly 1.19 million tonnes and foreign exchange earnings of Rs. 6082 crores for the year 2020-21 through the export of castor oil and derivatives, castor seed and cake (SEA, 2021). India is a monopoly in the global castor oil industry. In Telangana, castor production in 2020-21 was 0.03 lakh tonnes with the productivity of 355 Kg/ha. The major districts growing castor in Telangana are Narayanpet, Mahabubnagar, Gadwal and Wanaparthy covering an area of 11,727 acres (<http://www.agri.telangana.gov.in/>).

Castor oil, derived from castor seed is a source of vegetable oil with medicinal properties and many

industrial applications. The oil is basically a triglyceride which contains approximately 90 percent fatty acid chains of ricinoleate, oleate and linoleate are the other components (Deshamukh *et al.*, 2018). Castor being a sole source of ricinoleic acid (85-94%), 18 carbon monounsaturated fatty acid it assumes a lot of economic importance over other vegetable oils and differs from other oils because of its high specific gravity and thickness. Castor oil is used in the production of pharmaceuticals, lubricants, hydraulic and brake fluid, polymer materials, coating and fertilizer and is a desirable lubricant component because of its high viscosity across a wide temperature range (Severino *et al.*, 2012).

Castor is a hardy plant, requires low input, tolerates marginal soils, is easy to establish in the field and is resistant to drought. But there are various challenges that make castor crop cultivation difficult to pursue. Climate adaptability is one of the challenges restricting castor plantation. Labour-intensive harvesting process, susceptibility to pests and diseases and fluctuating market prices are the major barriers to its widespread cultivation.

## IMPACT OF CHEMICAL MUTAGENESIS USING ETHYL METHANE SULPHONATE

The major challenge for castor production is the development of high-yielding varieties which are non-shattering, dwarf, resistant or tolerant to disease and insect pests, low in ricin, ricinin and RCA content. These characteristics could be altered to enable widespread commercial production and turn it into a high-income cash crop with significant economic importance. The sources of variability required for this crops improvement are extremely restricted. As a result, conventional breeding procedures were not expected to make much advances. Induced mutations may generate new variability contributing to the crops high yield potential.

is well suited for rainfed and irrigated conditions and is resistant to *Fusarium* wilt and leaf hopper. The chemical Ethyl methane sulphonate is obtained from Hi media bio sciences. The seeds were surface sterilised with 1% per cent sodium hypochlorite solution for 10 min and rinsed with running water for three times. The surface sterilised seeds were soaked in sterilised distilled water for 24 h at room temperature. The seeds were then partitioned into smaller batches (containing about 30 seeds each). The seed was then transferred to aqueous solutions of varying doses of EMS and incubated for varying time periods at room temperature after which the treated seed were rinsed under running tap water for 1h in order to remove the excess EMS

### Details of the Treatments

<b>Number of treatments</b>			:	20		
T1	:	C1D1	T11	:	C3D3	
T2	:	C1D2	T12	:	C3D4	
T3	:	C1D3	T13	:	C4D1	
T4	:	C1D4	T14	:	C4D2	
T5	:	C2D1	T15	:	C4D3	
T6	:	C2D2	T16	:	C4D4	
T7	:	C2D3	T17	:	C5D1	
T8	:	C2D4	T18	:	C5D2	
T9	:	C3D1	T19	:	C5D3	
T10	:	C3D2	T20	:	C4D4	
<b>Factor 1 (C)</b>		<b>EMS concentration (5)</b>	<b>Factor 2 (D)</b>		<b>Duration of treatment (4)</b>	
<b>C1</b>	:	EMS 0.25%	<b>D1</b>	:	4 h	
<b>C2</b>	:	EMS 0.50%	<b>D2</b>	:	8 h	
<b>C3</b>	:	EMS 1.0%	<b>D3</b>	:	12 h	
<b>C4</b>	:	EMS 1.50	<b>D4</b>	:	16 h	
<b>C5</b>	:	CONTROL				
<b>Number of replications</b>			:	3		

### MATERIAL AND METHODS

The experiment was conducted in the year 2020-2021 at the Department of Plant Pathology, ICAR-IIOR, Rajendra nagar, Hyderabad. The seeds of popular castor hybrid DCH-519 was used in the experiment. The cultivar is a medium duration type and

and enable safe handling. The untreated seeds served as control.

The laboratory test for mutagenic effect on germination was conducted as per earlier procedures adopted by Tepora (1994) in castor and Ariraman *et al.* (2014) in red gram by using between paper

method. Three replications of 10 seeds in each treatment including control were uniformly placed on germination paper and the paper were rolled with plastic paper covering sheet to maintain moisture content. The rolls were placed in an upright position and incubated in germination chamber maintained at  $25 \pm 1^\circ\text{C}$  temperature with relative humidity of  $80 \pm 1\%$  (Fig 1).

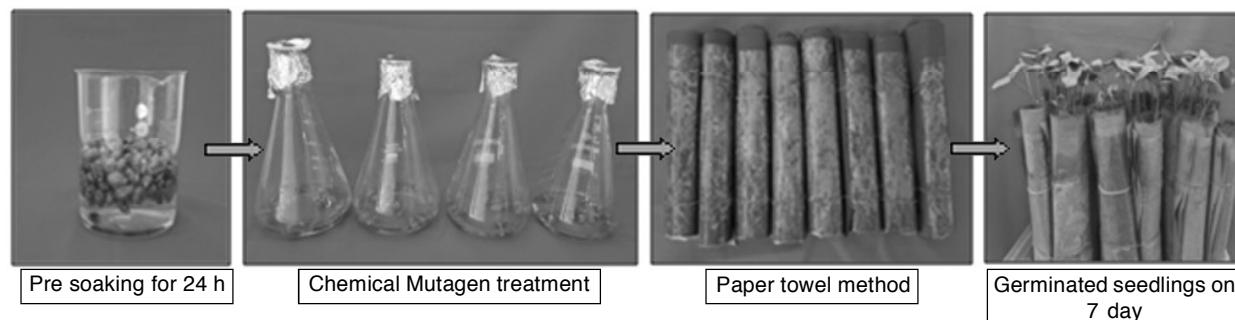


Figure 1: Flow chart showing steps followed in chemical mutagen treatment

### Seed germination per cent

The growth parameters were calculated as per ISTA rules. The evaluation of normal seedlings, abnormal seedlings, fresh un germinated seeds and dead seeds were done on seventh day. Germination percentage was expressed based on the number of normal seedlings and it was calculated as follows  
Germination (%) =

$$\frac{\text{Number of normal seedlings} \times 100}{\text{Total number of seeds planted}}$$

### Seedling length (cm)

The normal seedlings were selected randomly per replication in each treatment on 7th day of germination test. The root length was measured from the tip of the primary root to the base of the hypocotyl with the help of a scale and mean root length was expressed in centimeters. The normal seedlings used for root length measurement were also used for the measurement of shoot length. The shoot length was measured from the tip of the primary leaf to the base of the hypocotyl and mean shoot length was expressed in centimeters.

### Seedling dry weight (g)

The normal seedlings used for root and shoot length measurements were put into butter paper bags and kept in a hot air oven at  $80 \pm 1^\circ\text{C}$  for 24 hr. The mean dry weight of the seedlings were recorded and expressed in grams(g).

### Seedling vigour index I

Seedling vigour index I was calculated using the formula given by Abdul Baki and Anderson (1973) and expressed in whole number.

$$\text{Seedling vigour index I} = \text{Germination percentage (\%)} \times \text{Seedling length (cm)}$$

### Seedling vigour index II

Seedling vigour index II was calculated as per the method suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

$$\text{Seedling vigour index II} = \text{Germination percentage (\%)} \times \text{Seedling dry weight (g)}$$

### Statistical Analysis

The data recorded were analyzed statistically by adopting Two Factorial Completely Randomized Design (CRD) as described by Panse and Sukhatma (1985) and the standard error of difference was calculated at 5% probability level to compare the mean difference among the treatments. The data recorded as percentage were transformed to the respective angular (arc sin) values before subjecting them to statistical analysis.

## RESULTS AND DISCUSSION

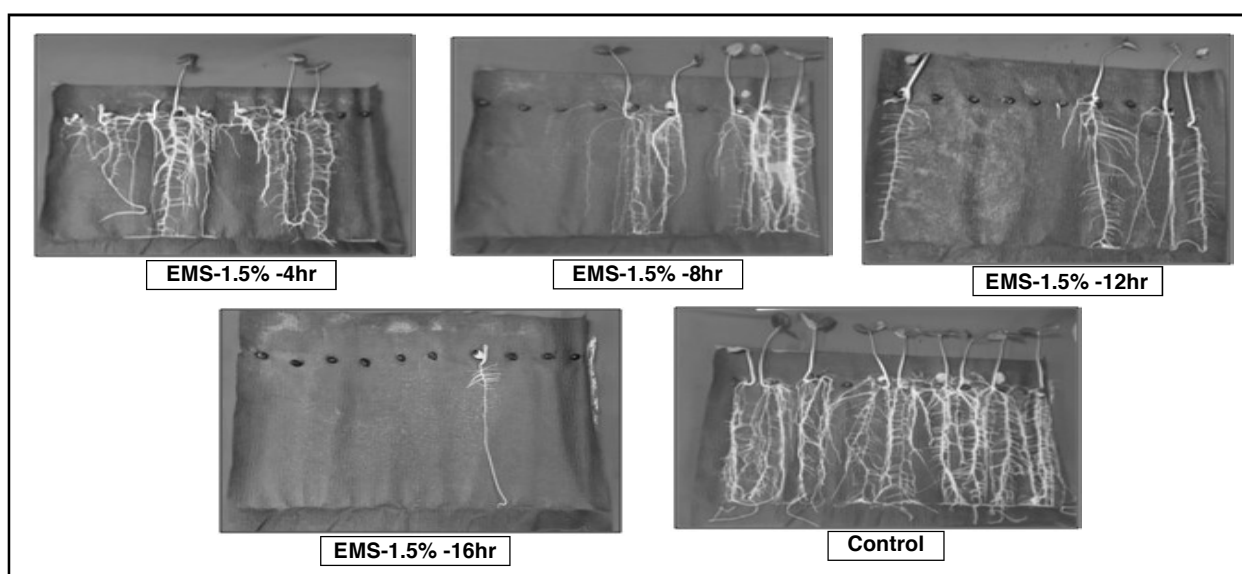
### Effect of Chemical mutagen (EMS) treatment on castor seed germination (%)

The highest seed germination was observed in control (90 %). The germination was decreased with the increase in the concentration of EMS from 0.25 to 1.5% and increase in treatment duration. The germination rate of castor seeds under different concentrations was significantly lower as compared to the control and the lowest germination percent (3.3%) was observed at 1.5% of EMS treated for 16h (Table 1 and Figure 2). This was in conformity with the earlier

**Table 1. Effect of chemical mutagen Ethyl methane sulphonate on seed germination of castor**

Treatment time Concentration of EMS (%)	Germination percentage (%)			
	4h	8h	12h	16h
0.25%	73.33 (58.98)*	76.67 (61.19)	83.33 (66.11)	70.00 (56.76)
0.50%	76.67 (61.12)	80.00 (63.40)	10.00 (18.42)	76.67 (61.12)
1.00%	76.67 (61.19)	76.67 (61.12)	53.33 (46.89)	30.00(33.19)
1.5%	76.67 (61.19)	53.33 (46.90)	46.67 (43.07)	3.33 (10.33)
control	86.67 (68.82)	83.33 (65.92)	86.67 (68.63)	90.00 (71.53)
	<b>Concentration (A)</b>	<b>Treatment time (B)</b>	<b>Concentration × Treatment time</b>	
C.D. at 5%	3.175	2.840	6.350	
SE(d)	1.565	1.400	3.130	
SE(m)	1.107	0.990	2.214	
C.V %	5.854			

\* Values in parenthesis are the angular transformed values



**Figure 2. Effect of chemical mutagen (Ethyl methane sulphonate) on germination of castor**

findings of Tepora *et al.* (2010) and Liu *et al.* (2015) in castor and reported that the seed germination percentage, germination index and speed of germination decreased as the concentration of EMS was increased.

The results were supported by the earlier works of Andries *et al.*, (2021) in Tepary bean and Vikhe and Nehul (2020) in *Vignaradiata*. In pigeon pea, an inhibitory effect on seed germination was clearly visible after the mutagenic treatments. From lower to

higher doses of mutagenic treatments, there was a steady decrease in germination (Sunil *et al.*, 2011). Similar results were reported by Datiret *et al.* (2007) in horse gram and Potdukhe and Narkhede (2002) in pigeon pea.

Germination of the treated plants showed a clear dosage rate relationship which was reduced as the concentrations of mutagenic treatments increased. The influence of mutagens on the seeds meristematic tissues may have caused the percentage reduction /

stimulation in seed germination. Seed germination may be affected by changes at the cellular level induced by physiological and acute chromosomal damage at greater mutagen concentrations. (Singh, *et al.*, 2011; Nilan, *et al.*, 1976.; Sinha and Godward, 1973). Chromosomal abnormalities such as delay in the onset of mitosis (Yadav, 1987) and aberrations induced catalase and lipase enzyme activity as well as hormone activity which resulted in decreased germination. One of the physiological impact of EMS that leads to a decrease in germination is disruption in the production of enzymes involved in the germination process.

The LD50 (Lethal dose) value was determined based upon the seed germination percentage. The 50 percentage seed germination was observed in 1%EMS treated for a duration of 12 hrs well as in 1.5% EMS for a duration of 8h.

#### Effect of Chemical mutagen (EMS) treatment on castor seedling length

The results on the effect of chemical mutagen treatment on seedling growth parameters of castor are shown in Table 2. With the increase in concentration of chemical mutagen a decrease in the seedling length was observed indicating a negative correlation between

**Table 2. Effect of chemical mutagen Ethyl methane sulphonate on castor seedling length**

Concentration of EMS (%)	Treatment Time (h)	Root length (cm)	Shoot length (cm)	Seedling length (cm)
0.25%	4	24.08	11.61	35.69
	8	20.06	11.17	31.23
	12	19.86	11.15	31.01
	16	22.61	9.74	32.33
0.5%	4	22.54	11.30	33.84
	8	23.08	10.76	33.85
	12	9.17	5.33	14.67
	16	19.56	9.37	28.93
1.0%	4	22.01	10.03	32.04
	8	23.65	10.33	33.97
	12	19.67	9.73	29.40
	16	21.49	11.03	32.52
1.5%	4	18.91	7.32	26.23
	8	22.14	10.69	32.83
	12	19.30	8.03	27.28
	16	4.00	1.50	5.50
	4	24.90	11.74	36.64
Control	8	24.93	12.83	37.77
	12	25.43	12.05	37.42
	16	25.57	12.70	38.27
	C.V %	7.815	3.316	2.797
Concentration(A)	C.D at 5%	1.33	0.27	1.39
	SE(d)	0.65	0.13	0.68
	SE(m)	0.46	0.09	0.48
Time of treatment (B)	C.D at 5%	1.19	0.24	1.25
	SE(d)	0.58	0.12	0.61
	SE(m)	0.41	0.08	0.43
Factor(A X B)	C.D at 5%	2.67	0.54	2.79
	SE(d)	1.31	0.26	1.37
	SE(m)	0.93	0.19	0.97

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dosage and the seedling length. The maximum seedling length observed was 35.69cm in T1 (0.25% concentration for 4 h) and the minimum observed was 5.50 cm in T16 (1.5% concentration for 16 h). Seedling length in control plants was 38.27cm. The gradual decrease in seedling length was recorded with an increase in the concentration of EMS.

Jayakumar and Selvaraj (2003) observed similar results in sunflower. Aparna *et al.* (2013) in groundnut, Talebi *et al.* (2012) in paddy, Borzouei *et al.* (2010) in wheat. Mutagenic sensitivity can be attributed to the level of differentiation of rudimentary plant parts at the time of treatment (Mahalle *et al.*, 2018)

The reduction in root and shoot length with increasing concentration of EMS demonstrated the inhibitory effect of mutagens on seedling length in *Vignaradiata* (Vikhe and Nehul, 2020). The effects of mutagens on the physiological system were linked to the reduction in root and shoot length (Gaul, 1977). A reduction in root and shoot length resulting from mutagenic treatments had previously been documented

in crop plants (Amarnath and Prasad, 1998). The stimulatory effect on the length of root, shoot and seedling was observed in lower concentrations of EMS. The stimulations caused by EMS treatments were thought to be caused by an increase in cell division rates as well as activation of growth hormone, such as auxin or inhibition of auxin synthesis (Zaka *et al.*, 2004). The increase in growth promoters, decrease in growth inhibitors and the decline of the absorption process may all contribute to lower growth as a result of high dosage. (Rajib and Jagatpati, 2011).

**Effect of Chemical mutagen (EMS) treatment on castor seedling vigour index:**

Seedling vigour which facilitate in categorizing strong and weak seedling and represent potential of seedling for successful establishment. The data pertaining to the effect of chemical mutagen treatment and treatment time on seedling vigour index-I and II are presented in the (Table 3). The highest mean of SVI- I (2612.26) was recorded in T1 (0.25% concentration for 4 hr) and minimum SVI-I (18.67) was found in T16 (1.5% concentration for 16 hr) whereas

**Table 3. Effect of chemical mutagen Ethyl methane sulphonate on seedling vigour index (SV-I and SV-II) of castor**

Concentration of EMS (%)	Treatment Time (hr)	Vigour index –1	Vigour index-2
0.25%	4	2612.26	53.49
	8	2401.70	53.73
	12	2583.43	52.1
	16	2263.10	47.3
0.5%	4	2595.53	51.99
	8	2708.26	53.33
	12	146.66	6.367
	16	2222.12	55.35
1.0%	4	2456.93	55.067
	8	2603.46	45.59
	12	1567.00	45.18
	16	975.53	58.54
1.5%	4	2010.80	53.7
	8	1750.66	48.76
	12	1273.41	36.05
	16	18.67	0.25

Control	4	3175.40	64.84
	8	3147.50	66.09
	12	3242.43	66.77
	16	3444.00	66.97
	C.V%	4.798	2.159
Concentration(A)	C.D. at 5%	85.82	0.878
	SE(d)	42.31	0.433
	SE(m)	29.91	0.306
Time of treatment (B)	C.D. at 5%	76.76	0.785
	SE(d)	37.84	0.387
	SE(m)	26.75	0.274
Factor(A X B)	C.D. at 5%	171.65	1.75
	SE(d)	84.61	0.86
	SE(m)	59.83	0.61

the untreated has shown a highest SVI- I (3444). The gradual reduction in root and shoot length with increase in concentration resulted in corresponding decrease in seedling vigour index. The highest SV-II (58.54) was recorded in T12 (1.0% concentration for 16 hr) and the lowest SV-II (0.25) was observed in T16 (1.5% concentration for 16 hr). Whereas the control has shown the highest SV-II of 66.97.

Seedling vigour index decreased with the increase in the concentration of EMS. It indicates the relative sensitivity of castor for varying dose of mutagenic treatments resulting on overall vigour of seedling and subsequent establishment of mutants. Similar results were reported by Aparna *et al.* (2013) in groundnut. It was also in conformity with the earlier reports of Sadashiv *et al.*, (2012) in horsegram, Hemavathy (2015) and Rukesh *et al.*, (2017) in green gram. Variations in diverse morphological features were most likely generated by mutagen EMS-induced phenotypically constructive multidirectional polygene mutations. The varied sensitivity of distinct loci among the genotype for different features could explain the differing responsiveness of seed following EMS treatments (Khokhar, 1998).

## CONCLUSION

The results of this study indicated that the germination of castor seeds was affected with increase in concentration/ treatment duration of EMS. Soaking of seeds at 1.5% EMS solution for 12 h was found to

be ideal for mutagenesis in castor as this treatment combination killed only 50% of the treated seeds. However, a firm conclusion regarding this observation will require empirical evidence from large-scale seed mutagenesis of diverse castor germplasm.

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## PERFORMANCE OF MACHINE PLANTED CHICKPEA (*Cicer arietinum* L.) AS INFLUENCED BY DIFFERENT SEED RATES AND NUTRIENT MANAGEMENT PRACTICES IN SOUTHERN TELANGANA ZONE

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### ABSTRACT

A field experiment was conducted in vertisols of Agricultural Research Institute, Main farm, Professor Jayashankar Telangana State Agricultural University, Hyderabad during *rabi* 2020-21 and 2021-22 to study response of chickpea (JG-11) to different seed rate with CIAE planter and nutrient management. The experimental plots were laid out in split plot design with different seed rates (4) as main plots and nutrient management as sub-plots (7). Application of 105 kg ha<sup>-1</sup> resulted in highest dry matter production (5296 and 5742 kg ha<sup>-1</sup>), seed yield (2548 and 2615 kg ha<sup>-1</sup>) and haulm yield (2764 and 3054 kg ha<sup>-1</sup>) among the seed rate treatments during 2020-21 and 2021-22 respectively. Among the nutrient management practices, application of 125 % RDF along with soil application of microbial consortia (N – *Azotobacter* + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup> recorded highest dry matter (5359 and 5606 kg ha<sup>-1</sup>), seed (2522 and 2640 kg ha<sup>-1</sup>) and haulm yield (2663 and 2936 kg ha<sup>-1</sup>) during 2020-21 and 2021-22 respectively.

**Keywords:** Chickpea, seed rate, nutrient management, growth and yield.

Chickpea (*Cicer arietinum* L.) is an important *rabi* pulse crop of India accounts for about 50 % of the total pulse production is an important source of vegetarian protein (20-22 %) and superior to other pulses. The area, production and productivity of chickpea during 2020-21 in India were 10 M ha, 11.9 M t and 1192 kg ha<sup>-1</sup> respectively in India (www.indiastat.com). In Telangana, it was cultivated in an area of 1.43 l ha with a production of 2.38 l t and productivity of 1667 kg ha<sup>-1</sup> during 2020-21 (www.indiastat.com). Most popular *desi* type chickpea cultivars in Telangana include ICC 37, JG 11, JG 130 and JAKI 9218.

Chickpea remains a crucial contributor to crop diversification for agricultural sustainability. It is well adapted to abiotic stresses and commonly cultivated in rainfed areas on residual soil moisture. Despite the silent revolution, there is still a great scope in Telangana state to expand area under chickpea for sustaining farmer's income and state's nutritional security in marginal lands.

In Telangana state, chickpea crop is mostly sown by broadcasting, wherein weeding is difficult and thus, results in reasonable yield loss. Mechanised sowing assumes greater importance in *rabi* crops due to the narrow sowing window after harvest of the preceding *kharif* crops. Further, delayed sowing has drastic effects on growth and yield of chickpea as it is mainly grown on residual soil moisture. Recommended seed rate differs for mechanical sowing based on cultivar (Sujathamma and Babu, 2019). Further, the rate of fertilizer application also needs to be redesigned in accordance to the seed rate. Application of biofertilizers in chickpea enhances the crop yield and quality by fixing the atmospheric nitrogen and improving the availability of native soil nutrients besides maintaining the soil fertility (Uddin *et al.*, 2014). Hence, there is a need for standardization of optimum seed rate and nutrient management practice under machine planted chickpea for achieving better growth and higher yield.

## MATERIAL AND METHODS

A field experiment was conducted in vertisols of Agricultural Research Institute, Main Farm, Professor Jayashankar Telangana State Agricultural University, Hyderabad during *rabi* 2020-21 and 2021-22, located at Southern Telangana Zone. The initial soil physico-chemical properties were determined following standard procedures. The soil of the experimental site was slightly alkaline in nature (pH-8.31), non saline (EC, 0.191  $\text{dsm}^{-1}$ ), low in organic carbon (0.37 %), low in available nitrogen (176  $\text{kg ha}^{-1}$ ), high in available phosphorus (73  $\text{kg ha}^{-1}$ ), high in available potassium (523  $\text{kg ha}^{-1}$ ) and medium in available zinc (0.99 ppm). The variety selected for the experimental study was JG-11, a ruling variety in Telangana. A total rainfall of about 18.86 mm was received during 2020-21 and 3.3 mm was recorded during 2021-22.

The experimental plots were laid out in split plot design with 4 main plots (Seed rate) *viz.*,  $M_1 - 52 \text{ kg ha}^{-1}$ ,  $M_2 - 70 \text{ kg ha}^{-1}$ ,  $M_3 - 77 \text{ kg ha}^{-1}$  and  $M_4 - 105 \text{ kg ha}^{-1}$  and 7 sub plots (Nutrient management practices) *viz.*,  $S_1$ - Absolute Control,  $S_2$ - 75 % RDF,  $S_3$ - 100 % RDF,  $S_4$ -125 % RDF,  $S_5$ - 75 % RDF + Soil application of Microbial consortia (N – *Azotobacter* + Phosphorus Solubilising Bacteria (PSB) + Potassium Releasing Bacteria (KRB)+ Zinc solubilising Bacteria (ZnSB) @ 5  $\text{kg ha}^{-1}$ ,  $S_6$ - 100 % RDF + Soil application of Microbial consortia (N – *Azotobacter* + PSB + KRB+ ZnSB) @ 5  $\text{kg ha}^{-1}$  and  $S_7$ - 125 % RDF + Soil application of Microbial consortia (N – *Azotobacter* + PSB + KRB+ ZnSB) @ 5  $\text{kg ha}^{-1}$ . All the treatments were replicated thrice. The treatments  $M_1$  and  $M_2$  were sown by adopting spacing of 45 cm x 10 cm and 45 cm x 7.5 cm while,  $M_3$  and  $M_4$  were sown at 30 cm x 10 cm and 30 cm x 7.5 cm respectively.

The recommended dose of fertilizer (RDF) is 20: 50: 20 N,  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$   $\text{kg ha}^{-1}$ . Entire dose of P and K along with 50 % dose of N was applied as basal while, remaining 50 % dose of N was applied as top dressing at 30 DAS. Source for N,  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  were urea, single super phosphate and muriate of potash respectively. Microbial consortia (N-*Azotobacter* + PSB + KRB + ZnSB) was mixed @ 5  $\text{kg ha}^{-1}$  along with 250 kg vermicompost and spread uniformly throughout the plots as basal. Regarding the seed rate treatment imposition, CIAE planter was

calibrated in the lab for the desired seed rate with two different plates *viz.*, 18 sleeves (for 7.5 cm inter row spacing) and 16 sleeves (for 10 cm inter row spacing). The planter used for sowing had incline plate metering type and inverted T type tyne manufactured by Central Institute of Agricultural Engineering, Bhopal.

Basal application of 75 kg of vermicompost was done uniformly to all experimental plots along with 750 g of *Rhizobium*, 750 g of *Trichoderma viridae* and 750 g of *Pseudomonas sp.* to ensure control against fungal diseases. Pre-emergence application of pendimethalin @ 1  $\text{kg a.i ha}^{-1}$  2 DAS was done during 2020-21. During 2021-22, tank mixture of pendimethalin and imazethapyr @ 1  $\text{kg a.i ha}^{-1}$  2 DAS after sowing was done. In plots with row spacing of 45 cm, weeding was done with the help of power weeder while, in those plots wherein row spacing was 30 cm, weeding was carried out using wheel hoe at 20 and 40 DAS. First irrigation was scheduled immediately after sowing to ensure uniform emergence. After that two irrigations were scheduled at critical stages of the crop (pre-flowering and pod formation) to ensure optimum moisture for better growth and yield. Recommended plant protection measures were taken up. Harvesting was done using mechanical reaper. Threshing was done manually using stripping method.

All the biometric parameters were recorded on five randomly selected representative plants. Destructive sampling for dry matter production was done from the rows earmarked next to the border rows. Seed and haulm yield were recorded from net plot area and used to work out yield per hectare.

Branch growth rate was calculated using the formula (Ramesh *et al.*, 2021),

Branch growth rate (branches  $\text{day}^{-1}$ ) =

$$\frac{N_1 - N_2}{t_1 - t_2}$$

where,

$N_1$  = number of branches at time  $t_1$

$N_2$  = number of branches at time  $t_2$

All the data collected during both years of experimentation pertaining to the parameters presented below were subjected to statistical scrutiny

by analysis of variance technique for split plot design suggested by Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

### Growth Parameters

#### Plant Height (cm)

Significant differences were observed in plant height due to varying seed rate and nutrient management practices during both the years of experimentation. However, the interaction of planting density and nutrient management was not significant on plant height during both the years of experimentation (Table 1).

During 2020-21 and 2021-22, significantly higher plant height was observed with seed rate of 105 kg ha<sup>-1</sup> (36.8 and 38.4 cm) which remained at par with seed rate of 77 kg ha<sup>-1</sup> (35.4 and 37.1 cm) and superior over the rest of the treatments. The mean data of 2 years also followed the same trend as that of 2020-21 and 2021-22.

The increase in plant height with increasing seed rate might be attributed to the interplant competition for light and space. Similar results with respect to increased plant height with an increase in seed rate in chickpea were earlier reported by Singh *et al.*, 2017.

With regard to nutrient management practices, significantly higher plant height was recorded with the application of 125 % RDF + MC (38.6 and 39.6 cm) and remained statistically at par with 125 % RDF (36.8 and 38.7 cm) and 100 % RDF + MC (35.8 and 38.1 cm) and remained significantly superior to all other treatments respectively. The mean of two years also followed similar trend as that of individual years of experimentation (2020-21 and 2021-22).

Taller plants observed with crop imposed with combined application of 125 % RDF + microbial consortia could be attributed to the conjunctive nutrient sources and favorable soil nutrient status that helped towards slow release of the nutrients throughout crop growth period and enhanced cell multiplication and elongation in comparison to corresponding lower level of fertilizer. Meena *et al.* (2020) also reported significant improvement in plant height with

conjunctive application of microbial consortia and inorganic chemical fertilizers in chickpea.

#### Number of branches plant<sup>-1</sup> and branch growth rate (branches day<sup>-1</sup>)

Number of branches plant<sup>-1</sup> are determinant of number of pods in chickpea crop that ultimately decide the crop yield. Number of branches plant<sup>-1</sup> were significantly influenced by the seed rate as well as the nutrient management during both the years of experimentation.

Seed rate of 52 kg ha<sup>-1</sup> (26.4, 27.8 and 27.1) recorded significantly higher number of branches over all other seed rate treatments during 2020-21, 2021-22 and mean of 2 years respectively. Branch growth rate was significantly higher with the seed rate of 52 kg ha<sup>-1</sup> (0.67) which remained at par with 70 kg ha<sup>-1</sup> (0.63 and 0.64) and superior over rest of the treatments during 2020-21 and 2021-22 respectively. The mean of 2 years (2020-21 and 2021-22) revealed that the seed rate of 52 kg ha<sup>-1</sup> recorded significantly superior branch growth rate (0.69) over all other seed rate *viz.*, 70 (0.63), 77 (0.51) and 105 kg ha<sup>-1</sup> (0.48).

Improved branching under 52 kg ha<sup>-1</sup> could be ascribed to the lower plant density (number of plants m<sup>-2</sup>) that increased the space available per plant resulting in less competition for resources *viz.*, nutrients, water, light and space. Further, lower seed rate might have allowed more sunlight to penetrate the canopy and improved vegetative growth and reflected in higher number of branches. Aggarwal *et al.*, (2016) also documented similar results of decrease in number of branches with higher planting density in chickpea.

Similarly, among nutrient management practices during both years of experimentation (2020-21 and 2021-22), 125 % RDF + MC resulted in significantly higher number of branches (24.1 and 26.4) and remained superior to all other treatments except 125 % RDF (25.9 and 28.2) respectively. The mean of 2 years also followed the same trend as during 2020-21 and 2021-22.

Nutrient management practices also significantly influenced branch growth rate during both years (2020-21 and 2021-22). Application of 125

% RDF + MC resulted in significantly higher branch growth rate (0.64 and 0.67) over 75 % RDF (0.53 and 0.54) and absolute control (0.48 and 0.48) and remained statistically at par with all other treatments during 2020-21 and 2021-22 respectively.

Higher number of branches could be attributed to the adequate nutrient supply to the crop in different nutrient management treatments as compared to the control plot wherein, the crop was deprived of the nutrients and resulted in poor and stunted growth. Similar findings on increased number of branches in chickpea with conjunctive application of inorganics and microbial inoculation were earlier documented by Namvar *et al.* (2011) and Nawange *et al.* (2018).

The interaction between seed rate and nutrient management was non-significant during both the years of experimentation.

#### **Dry matter production (kg ha<sup>-1</sup>)**

During both the years of experimentation, dry matter production was significantly influenced by seed rate as well as nutrient management. Interaction between seed rate and nutrient management remained non-significant during 2020-21 while, it was significant during 2021-22 (Table 1).

During both the years, application of 105 kg ha<sup>-1</sup> seed rate recorded significantly higher dry matter (5296 and 5642 kg ha<sup>-1</sup>) over all other treatments during 2020-21 and 2021-22 respectively.

Improved dry matter production with seed rate of 105 kg ha<sup>-1</sup> could be ascribed to the higher plant population coupled with more number of branches and leaf area, which in turn contributed to high photosynthesis and dry matter production in comparison to corresponding lower seed rate of 52, 70 and 77 kg ha<sup>-1</sup>. Similar results on increased dry matter production with higher seed rate in chickpea crop were reported by Kamithi *et al.*, 2009.

During 2020-21, among the nutrient management practices, application of 125 % RDF + MC recorded significantly higher dry weight (5359 and 5606 kg ha<sup>-1</sup>) and remained statistically at par with the application of 125 % RDF (5178 and 5451 kg ha<sup>-1</sup>) and 100 % RDF + MC (5013 and 5276 kg

ha<sup>-1</sup>) and superior to all other treatments. Mean data of dry matter production followed a similar trend as that of individual years.

The increase in dry matter production at higher fertility levels might be due to improved growth parameters *viz.*, plant height and number of branches in the respective treatments. These results corroborate with those of Meena *et al.* (2020) who documented increased dry matter production with increase in fertility level in chickpea crop.

#### **Yield attributes and yield**

##### **Number of pods plant<sup>-1</sup>**

Yield of a crop is dependent on the yield attributes and the variation in yield attributes help us to understand the behavior of individual crop in response to treatments imposed. An overview of data clearly indicated that number of pods plant<sup>-1</sup> were significantly influenced by the seed rate and nutrient management during both 2020-2021 and 2021-22. However, the interaction effect of seed rate and nutrient management was found to be non-significant on number of pods plant<sup>-1</sup> during both the years of experimentation.

Among the seed rate treatments, 52 kg ha<sup>-1</sup> produced significantly higher number of pods plant<sup>-1</sup> (45.2 and 46.3) over 105 kg ha<sup>-1</sup> (41.1 and 41.3) during 2020-21 and 2021-22 respectively. Mean data of two years also followed a similar trend.

Optimum plant population had better growth resources (sunlight, water and nutrients) that reflected in higher leaf area and hence, more photosynthesis and assimilate translocation to sink (pod) from source (leaf). On the other hand, lower number of pods plant<sup>-1</sup> with higher seed rate might be due to competition for resources coupled with flower abortion. These results find support with the findings of Kumar *et al.* (2017) in chickpea.

With respect to nutrient management treatments, application of 125 % RDF + MC recorded significantly higher number of pods plant<sup>-1</sup> (46.7 and 48.1) over all other nutrient management practices except 125 % RDF (45.2 and 46.1) and 100 % RDF + MC (44.8 and 45.6) during 2020-21 and 2021-22 respectively.

Table 1. Growth parameters of chickpea as influenced by seed rate and nutrient management practices

Treatments	Plant height (cm)		Number of branches		Branch growth rate (branches day <sup>-1</sup> )		Dry matter production (kg ha <sup>-1</sup> )				
	2020-21	2020-21	2020-21	Mean	2020-21	2020-21	2020-21	Mean			
<b>Main Plot - Seed rate (M)</b>											
M <sub>1</sub> -52 kg ha <sup>-1</sup>	31.3	32.0	26.4	27.8	27.1	0.67	0.71	0.69	4279	4467	4373
M <sub>2</sub> -70 kg ha <sup>-1</sup>	32.6	33.9	24.4	25.7	25.1	0.63	0.64	0.63	4572	4859	4716
M <sub>3</sub> -77 kg ha <sup>-1</sup>	35.4	37.1	20.6	21.6	21.1	0.50	0.51	0.51	4858	5207	5033
M <sub>4</sub> -105 kg ha <sup>-1</sup>	36.8	38.4	19.2	20.4	19.8	0.48	0.48	0.48	5296	5642	5469
S.E.m±	0.49	0.62	0.69	0.64	0.62	0.01	0.02	0.01	105.4	58.6	56.5
CD (P=0.05)	1.71	2.16	2.40	2.20	2.14	0.05	0.08	0.05	364.8	202.9	195.4
<b>Sub plot – Nutrient management (S)</b>											
S <sub>1</sub> -Absolute control	27.2	28.3	19.3	19.4	19.3	0.48	0.48	0.48	3996	4459	4228
S <sub>2</sub> -75 % RDF	32.0	32.8	20.9	21.8	21.3	0.53	0.54	0.53	4452	4840	4646
S <sub>3</sub> -100 % RDF	35.3	36.7	22.7	23.7	23.2	0.58	0.59	0.58	462	5021	4857
S <sub>4</sub> -125 % RDF	36.8	38.7	24.1	26.4	25.2	0.61	0.63	0.62	5179	5451	5315
S <sub>5</sub> -75 % RDF + MC	32.6	33.4	22.2	23.0	22.6	0.56	0.58	0.57	4568	4927	4748
S <sub>6</sub> -100 % RDF + MC	35.8	38.1	23.5	24.7	24.1	0.60	0.62	0.61	5013	5276	5145
S <sub>7</sub> -125 % RDF + MC	38.6	39.6	25.9	28.2	27.1	0.64	0.67	0.66	5359	5606	5483
S.E.m±	0.96	0.98	0.87	0.97	0.82	0.04	0.04	0.04	200.2	80.6	107.6
CD (P=0.05)	2.73	2.77	2.48	2.75	2.32	0.10	0.10	0.10	569.4	229.2	306.0
<b>Interaction (M x S)</b>											
SE±	1.85	1.91	1.75	1.90	1.63	0.07	0.07	0.07	385.5	160.4	207.1
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	458.4	NS
<b>Interaction (S x M)</b>											
SE±	1.92	1.95	1.74	1.93	1.63	0.07	0.07	0.07	400.5	161.2	215.3
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	443.6	NS

Note: RDF- 20: 50: 20 N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O kg ha<sup>-1</sup>

MC - Microbial consortia (N –Azotobacter + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup>.

Branch growth rate is given for 30 -60 DAS (maximum growth stage).

Improved growth parameters registered with these treatments (Table 1) had directly contributed to high number of pods per plant. Meena *et al.* (2020) also reported improved yield attributes of chickpea with integration of synthetic fertilizers and microbial inoculants.

### Seed and haulm yield (kg ha<sup>-1</sup>)

During both the years of experimentation (2020-21 and 2021-22), seed and haulm yield were significantly influenced by seed rate and nutrient management treatments. The interaction effect of the two factors remained non-significant during 2020-21 and significant during 2021-22 for seed yield. However, haulm yield was non-significant due to the interaction effect during both the years.

Output of analysis of variance (Table 2) revealed that seed rate of 105 kg ha<sup>-1</sup> resulted in significantly to higher seed yield (2548 and 2615 kg ha<sup>-1</sup>) over all other treatments during 2020-21 and 2021-22 respectively. With respect haulm yield, higher seed rate of 105 kg ha<sup>-1</sup> resulted in production of significantly higher seed and haulm yield (2764 and 3054 kg ha<sup>-1</sup>) over all other treatments except 77 kg ha<sup>-1</sup> (2516 and 2702 kg ha<sup>-1</sup>). Increase in seed yield with seed rate of 105 kg ha<sup>-1</sup> was about 43.3 and 40.3 % over 52 kg ha<sup>-1</sup> during 2020-21 and 2021-22 respectively.

Although higher number of pods plant<sup>-1</sup> were registered with seed rate of 52 kg ha<sup>-1</sup> due to better resource availability and reduced interplant competition, significantly higher seed and haulm yield were produced with seed rate of 105 kg ha<sup>-1</sup>. The improvement in individual plant yield was not sufficient to compensate potential yield as that of increased plants per unit area at higher plant density with seed rate of 105 kg ha<sup>-1</sup>. Patil *et al.*, (2021) also reported a similar trend of increased yield with higher seed rate despite of the higher yield attributes at lower seed rate in chickpea.

During 2021-22, among the nutrient management practices, application of 125 % RDF + MC resulted in significantly higher seed yield (2522 and 2640 kg ha<sup>-1</sup>) followed by 125 % RDF (2379 and 2539 kg ha<sup>-1</sup>) and 100 % RDF + MC (2323 and 2436 kg ha<sup>-1</sup>) and remained superior to the other treatments. Conjunctive use of 125 % RDF and

microbial consortia resulted in increase of seed yield to the magnitude of 41.6 and 48.3 % over absolute control during 2020-21 and 2021-22 respectively.

Conjunctive use of 125 % RDF and microbial consortia resulted in increase of seed yield to the magnitude of 41.6 and 48.3 % over absolute control during 2020-21 and 2021-22 respectively.

With respect to haulm yield, significantly higher haulm yield was recorded with 125 % RDF + MC (2663 and 2936 kg ha<sup>-1</sup>) over 75 % RDF (2292 and 2448 kg ha<sup>-1</sup>) and absolute control (2036 and 2207 kg ha<sup>-1</sup>) while it remained statistically at par with rest of the treatments during 2020-21 and 2021-22 respectively. Mean data of seed and haulm yield followed a similar trend as that of individual years.

Combination of higher dose of fertilizer with microbial inoculants resulted in higher yield which could be ascribed to the fact that application of 125 % and 100 % RDF facilitated adequate amount of nutrients that favoured better growth and development of root system and nutrient uptake. Further, microbial inoculation helped in the process of nodulation, nitrogen fixation, root elongation, leaf expansion promoting better photosynthetic efficiency and growth of crop that reflected in improved seed and haulm yield. Nawange *et al.*, (2018) and Sangma and Changde, 2020 also documented improved seed and haulm yield of chickpea with higher level of nutrients in conjunction with microbial inoculation.

### Interaction effect of seed rate and nutrient management

Interaction effect of planting density and nutrient management was found to be significant on dry matter production and seed yield of chickpea during 2021-22 (Table 3 and 4). Seed rate of 105 kg ha<sup>-1</sup> with 125 % RDF + MC produced significantly higher dry matter (6271 kg ha<sup>-1</sup>) over all other treatment combinations except with seed rate of 105 kg ha<sup>-1</sup> along with 125 % RDF alone (6225 kg ha<sup>-1</sup>) and 100 % RDF + MC (5846 kg ha<sup>-1</sup>). With regard to seed yield also, seed rate of 105 kg ha<sup>-1</sup> along with 125 % RDF + MC produced significantly higher seed yield (3077 kg ha<sup>-1</sup>) followed by the combination 105 kg ha<sup>-1</sup> with 125 % RDF (3069 kg ha<sup>-1</sup>) and 105 kg ha<sup>-1</sup> with 100 % RDF + MC (2929 kg ha<sup>-1</sup>) while remained superior to all other treatment combinations.



Table 2. Yield attributes and yield of chickpea as influenced by seed rate and nutrient management practices

Treatments	2020-21			2021-22			Mean			Seed yield (kg ha <sup>-1</sup> )			Haulm yield (kg ha <sup>-1</sup> )		
	2020-21	2021-22	Mean	2020-21	2021-22	Mean	2020-21	2021-22	Mean	2020-21	2021-22	Mean	2020-21	2021-22	Mean
<b>Main Plot - Seed rate (M)</b>															
M1-52 kg ha <sup>-1</sup>	45.2	46.3	45.7	1784	1862	1823	2093	2224	2159						
M2-70 kg ha <sup>-1</sup>	44.1	45.0	44.6	2120	2200	2160	2348	2515	2431						
M3-77 kg ha <sup>-1</sup>	43.7	44.3	44.0	2214	2356	2285	2516	2702	2609						
M4-105 kg ha <sup>-1</sup>	41.1	41.3	41.2	2548	2615	2582	2764	3054	2909						
SE±	0.71	1.14	0.88	52.0	47.8	23.7	77.8	105.1	68.6						
CD (P=0.05)	2.46	3.95	3.03	179.9	165.5	82.1	269.4	363.6	237.4						
<b>Sub plot – Nutrient management (S)</b>															
S1-Absolute control	40.4	40.5	40.4	1776	1778	1777	2036	2207	2122						
S2-75 % RDF	41.9	42.3	42.1	1962	2038	2000	2292	2448	2370						
S3-100 % RDF	43.7	44.4	44.0	2120	2218	2169	2469	2662	2566						
S4-125 % RDF	45.2	46.1	45.6	2379	2539	2459	2642	2860	2751						
S5-75 % RDF + MC	42.1	42.7	42.4	2085	2158	2122	2394	2484	2439						
S6-100 % RDF + MC	44.8	45.6	45.2	2323	2436	2380	2515	2769	2642						
S7-125 % RDF + MC	46.7	48.1	47.4	2522	2640	2581	2663	2936	2799						
SE±	1.34	1.53	1.35	97.41	39.8	54.0	126.6	169.8	104.0						
CD (P=0.05)	3.80	4.35	3.85	277.0	113.1	153.6	360.0	482.9	295.8						
<b>Interaction (M x S)</b>															
SE±	2.57	3.05	2.65	187.7	87.8	102.8	247.0	331.6	204.5						
CD (P=0.05)	NS	NS	NS	NS	344.0	NS	NS	NS	NS						
<b>Interaction (S x M)</b>															
SE±	2.67	3.06	2.71	194.8	79.5	108.0	253.2	339.7	208.5						
CD (P=0.05)	NS	NS	NS	NS	226.1	NS	NS	NS	NS						

Note: RDF- 20: 50: 20 N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O kg ha<sup>-1</sup>  
 MC - Microbial consortia (N –Azotobacter + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup>

**Table 3. Interaction effect of planting density and nutrient management practices on dry matter production (kg ha<sup>-1</sup>) of chickpea at harvest during 2021-22.**

Treatments	Seed rate (kg ha <sup>-1</sup> )					
	Nutrient management	M1-52	M2-70	M3-77	M4-105	Mean
S1-Absolute control		3610	3980	4955	5291	4459
S2-75 % RDF		4237	4722	4993	5409	4840
S3-100 % RDF		4307	5027	5204	5549	5021
S4-125 % RDF		4871	5140	5567	6225	5451
S5-75 % RDF + MC		4253	4861	5092	5503	4927
S6-100 % RDF + MC		4842	4987	5429	5846	5276
S7-125 % RDF + MC		5145	5298	5711	6271	5606
Mean		4467	4859	5207	5742	
		SE±			CD (P=0.05)	
Main (M)		58.6			202.9	
Sub (S)		80.6			229.2	
Sub (S) at same main (M)		161.2			458.4	
Main (M) at same or different sub (S)		160.4			443.6	

Note: RDF (Recommended dose of fertilizer) 20: 50: 20 N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O kg ha<sup>-1</sup>  
 MC- Microbial consortia (N –Azotobacter + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup>

**Table 4. Interaction effect of planting density and nutrient management practices on grain yield (q ha<sup>-1</sup>) of chickpea during 2021-22.**

Treatments	Seed rate (kg ha <sup>-1</sup> )					
	Nutrient management	M <sub>1</sub> -52	M <sub>2</sub> -70	M <sub>3</sub> -77	M <sub>4</sub> -105	Mean
S <sub>1</sub> -Absolute control		1277	1692	2050	2092	1778
S <sub>2</sub> -75 % RDF		1725	2029	2068	2329	2038
S <sub>3</sub> -100 % RDF		1889	2228	2346	2408	2218
S <sub>4</sub> -125 % RDF		2092	2429	2565	3069	2539
S <sub>5</sub> -75 % RDF + MC		1823	2175	2236	2398	2158
S <sub>6</sub> -100 % RDF + MC		1947	2340	2529	2929	2436
S <sub>7</sub> -125 % RDF + MC		2280	2509	2696	3077	2640
Mean		1862	2200	2356	2615	
		SE±			CD (P=0.05)	
Main (M)		47.8			165.5	
Sub (S)		39.8			113.1	
Sub (S) at same main (M)		87.8			344.0	
Main (M) at same or different sub (S)		79.5			226.1	

Note: RDF (Recommended dose of fertilizer) 20: 50: 20 N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O kg ha<sup>-1</sup>  
 MC- Microbial consortia (N –Azotobacter + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup>

## PERFORMANCE OF MACHINE PLANTED CHICKPEA

Higher seed rate resulted in significantly higher dry matter production on unit area basis while, higher dose of fertilizer in combination with microbial inoculation resulted in better development of branches and leaf area that might have led to increased in dry matter production and yield attributes that reflected in higher seed yield of chickpea and these results are in line with those of Kamithi *et al.*, 2009.

### CONCLUSION

From the results of present study, it can be concluded that seed rate of 105 kg ha<sup>-1</sup> along with application of 125 % RDF + soil application of microbial consortia (N –*Azotobacter* + PSB + KRB+ ZnSB) @ 5 kg ha<sup>-1</sup> could be recommended to the farmers towards realization of better growth and yield of machine planted chickpea.

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## SCREENING OF F<sub>2</sub> POPULATION OF RICE FOR BACTERIAL BLIGHT IN RICE (*Oryza sativa* L.)

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### ABSTRACT

The bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most destructive diseases of rice in Asia. The disease can cause 20 to 80 percent yield loss and is difficult to manage by other means effectively. Development of host plant resistance is the most effective means to control this disease. Present study is an effort towards development of durable bacterial blight resistant varieties with high yield and as part of this, crossing was attempted between parents, IBTGM14 (resistant to gall midge and bacterial blight) and YPB46, (a high yielding line), F<sub>s</sub> were generated and confirmed. The F<sub>2</sub> population was screened against the pathogen under field condition by artificial inoculation. Among the two hundred and ninety five F<sub>2</sub> individual plants screened at College Farm, PJTSAU, during *Rabi* 2020-21, 82 plants were found to be resistant against bacterial blight. These resistant plants which can be forwarded to further generations and evaluated for yield performance in future.

**Key words:** Rice, bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, F<sub>2</sub>, Screening.

Rice (*Oryza sativa* L.) is one of the most important staple foods for human beings and is the most widely cultivated crop all over the world. India stands first in the area with 44.95 million hectares under rice cultivation and second in production with 121 million tonnes, constituting up to 21 % of global rice production (FAO, 2020). Rice is vulnerable to a number of diseases caused by bacteria, viruses, or fungi (Dai et al., 2010). Among the bacterial diseases, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in Asia. Bacterial blight (BB) is one of the oldest recorded rice diseases, which was first found by a farmer in the Fukuoka area of southern Japan in 1884 (Nino-Liu et al., 2006). Depending on the stage of infection and severity of the disease under natural conditions, the extent of loss due to bacterial blight disease has been reported to vary from 20-50 % (Singh et al., 2011). There are no effective chemical and biological bactericides available for controlling bacterial blight. Deployment of gene-conferred host plant resistance provides an economical, effective, environment friendly approach to control plant diseases and minimize the losses. At the Institute of

Biotechnology, PJTSAU, Hyderabad, efforts are underway towards development of biotic stress resistant high yielding rice varieties. As part of this goal, the present study involved phenotypic screening of an F<sub>2</sub> population against bacterial blight for the development of durable resistant varieties.

### MATERIAL AND METHODS

An F<sub>2</sub> population consisting of 295 individual plants along with parents (IBTGM14 and YPB46 and susceptible checks (MTU1010 and TN1) were raised as seedlings in nursery and were transplanted to main field with a spacing of 15 × 20cm, during *Rabi* 2020-21 at college Farm, PJTSAU, Rajendranagar, Hyderabad. Field preparation and all other recommended agronomic practices were followed except plant protection measures.

At 40 DAT, i.e., at maximum tillering stage, leaves of F<sub>2</sub> plants were inoculated with bacterial culture of *Xoo* strain IX-020, using leaf clipping method (Kauffman et al., 1973). Scissors sterilized with 70 % ethanol and dipped in bacterial suspension were used to inoculate the rice plants. Inoculation was done by cutting one fourth of top 3- 4 leaves of

## SCREENING OF $F_2$ POPULATION OF RICE FOR BACTERIAL BLIGHT IN RICE

the rice plant using these BB culture laden scissors. Data on BB incidence were taken at 15 days after inoculation using the scores as per Standard Evaluation System (SES) (IRRI, 2013) (Table 1).

be resistant with percent disease incidence ranging from 0.5 to 5 %, 142 plants were found to be moderately resistant with percent disease incidence ranging from

**Table1. Standard Evaluation System for Bacterial Blight scoring**

Score	% Disease incidence(DI)	Category
1	1-5%	Resistant
3	6-12%	Moderately resistant
5	13-25%	Moderately susceptible
7	26-50%	Susceptible
9	51-10%	Highly susceptible

SES, IRRI, 2013.

### RESULTS AND DISCUSSION

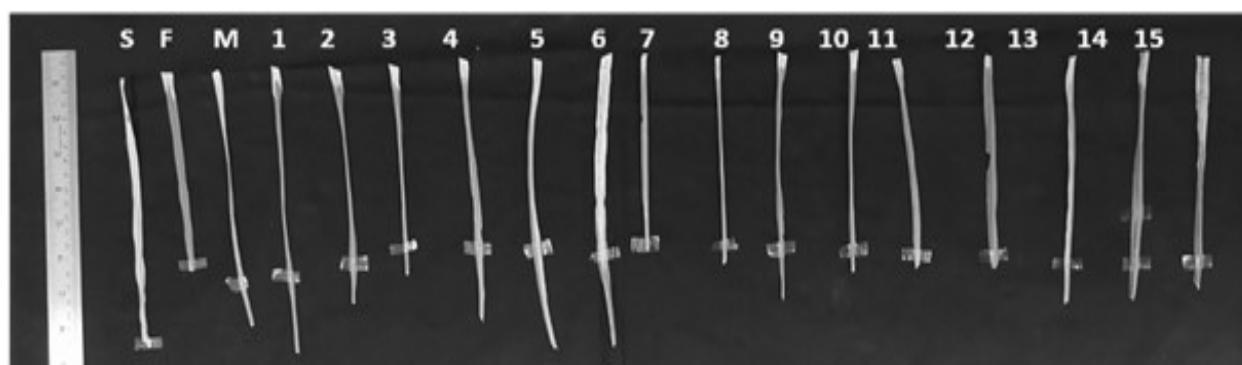
Two hundred and ninety five  $F_2$  individual plants were evaluated for bacterial blight resistance under field conditions at during *Rabi* 2020-21 at college Farm, PJTSAU, Rajendranagar, Hyderabad at 40 DAT and scores were recorded using SES, IRRI, 2013 scale at 15 days after inoculation. The susceptible checks, TN1 and MTU1010 showed highly susceptible reaction with percent disease incidence of 77.3% and 50.9% respectively as expected, as they were not carrying any of the bacterial blight resistance genes. Parents, IBTGM14 and YPB46 showed percent disease incidence of 3.2 and 32.7% respectively. This was expected as the female parent IBTGM14, carried *xa13* and *Xa21* bacterial blight resistance genes and male parent YPB46 does not carry any of the resistance genes. Out of the 295 plants, 88 plants were found to

6 to 12 %, where as all the remaining plants fell into susceptible category.

In the present study  $F_2$  plants were screened under field conditions for bacterial blight by following leaf clip method of inoculation and scores were recorded using IRRI, SES, 2013. The disease incidence of individual plants were recorded and classified the plants as resistant (score 1), moderately resistant (score 3), moderately

**Table 2. Classification of  $F_2$  population based on BB resistance**

Category	Number of entries
Resistant	88
Moderately resistant	142
Moderately susceptible	54
Susceptible	11



S:TN1; F:IBTGM14; M:YPB46; 1-4 : Resistant plants ( $F_2$ -29,49,70,120); 5: Moderately resistant ( $F_2$ -13), 6: Susceptible plants ( $F_2$ -65); 7-12: Resistant Plants ( $F_2$ -133, 233,235,241,253,258; 13-14: Moderately Resistant ( $F_2$ -256,257); 15: Moderately Susceptible (203)

**Figure 1. Disease reaction of  $F_2$  plants along with parents after inoculation with Xoo strain IX - 020**

Table 3. Screening of F<sub>2</sub> plants for bacterial blight resistance at college farm during Rabi 2020-21

F <sub>2</sub> Plant No.	%DI	Reaction	Score	F <sub>2</sub> Plant No.	%DI	Reaction	Score	F <sub>2</sub> Plant No.	%DI	Reaction	Score
1	6.2	MR	3	44	12.5	MS	3	87	9.6	MR	3
2	12.1	MS	5	45	3.0	R	1	88	3.3	R	1
3	6.8	MR	3	46	11.0	MR	3	89	9.2	MR	3
4	6.7	MR	3	47	35.5	S	9	90	12.6	MS	5
5	7.2	MR	3	48	6.5	MR	3	91	13.3	MS	5
6	6.5	MR	3	49	2.7	R	1	92	28.1	S	7
7	10.2	MR	3	50	6.8	MR	3	93	9.1	MR	3
8	12.7	MS	3	51	3.5	R	1	94	15.1	MS	5
9	12.3	MS	5	52	3.0	R	1	95	14.8	MS	5
10	12.3	MS	5	53	8.2	MR	3	96	8.2	MR	3
11	8.4	MR	3	54	4.5	R	1	97	8.5	MR	3
12	12.8	MS	5	55	17.3	MS	5	98	29.0	S	7
13	6.4	MR	3	56	4.4	R	1	99	28.3	S	7
14	5.9	MR	3	57	3.6	R	1	100	34.8	S	7
15	13.4	MS	5	58	4.5	R	1	101	4.1	R	1
16	12.6	MS	5	59	7.0	MR	3	102	5.4	MR	3
17	6.6	MR	3	60	17.6	MS	5	103	5.0	MR	3
18	3.0	R	1	61	7.4	MR	3	104	10.8	MR	3
19	5.7	MR	3	62	1.8	R	1	105	6.2	MR	3
20	6.7	MR	3	63	1.3	R	1	106	34.9	S	7
21	3.0	R	1	64	8.4	MR	3	107	7.9	MR	3
22	3.5	R	1	65	24.8	S	5	108	7.6	MR	3
23	11.8	MR	3	66	9.9	MR	3	109	12.0	MS	5
24	2.6	R	1	67	15.4	MS	5	110	6.4	MR	3
25	14.7	MS	5	68	13.2	MS	5	111	11.9	MR	3
26	11.3	MR	3	69	11.5	MR	3	112	6.5	MR	3
27	11.5	MR	3	70	2.7	R	1	113	10.7	MR	3
28	3.6	R	1	71	5.7	MR	3	114	6.7	MR	3
29	3.0	R	1	72	9.8	MR	3	115	9.3	MR	3
30	2.1	R	1	73	14.8	MS	5	116	19.2	MS	5
31	2.7	R	1	74	14.4	MS	5	117	2.8	R	1
32	6.8	MR	3	75	7.7	MR	3	118	2.7	R	3
33	6.9	MR	3	76	6.6	MR	3	119	11.4	MR	3
34	14.6	MS	5	77	6.4	MR	3	120	2.9	R	1
35	11.7	MR	3	78	11.8	MR	3	121	3.3	R	1
36	15.9	MS	3	79	8.0	MR	3	122	5.4	MR	3
37	6.1	MR	3	80	6.1	MR	3	123	10.1	MR	3
38	2.7	R	1	81	12.0	MR	3	124	4.6	R	1
39	3.9	R	1	82	2.7	R	1	125	11.4	MR	3
40	3.0	R	1	83	18.4	MS	5	126	9.6	MR	3
41	5.9	MR	3	84	10.4	MR	3	127	6.4	MR	3
42	8.3	MR	3	85	8.5	MR	3	128	2.6	R	1
43	2.8	R	1	86	9.8	MR	3	129	3.9	R	1

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$F_2$ Plant No.	%DI	Reaction	Score	$F_2$ Plant No.	%DI	Reaction	Score	$F_2$ Plant No.	%DI	Reaction	Score
130	10.6	MR	3	175	15.6	MS	5	220	2.7	R	1
131	11.1	MR	3	176	10.9	MR	3	221	11.4	MR	3
132	3.4	R	1	177	19.4	MS	5	222	10.3	MR	3
133	4.4	R	1	178	16.1	MS	5	223	2.4	R	1
134	10.7	MR	3	179	31.1	S	7	224	1.6	R	1
135	10.9	MR	3	180	42.7	S	7	225	2.2	R	1
136	12.8	MS	5	181	41.8	S	7	226	4.3	R	1
137	4.3	R	1	182	9.4	MR	3	227	2.7	R	1
138	11.1	MR	3	183	9.6	MR	3	228	2.5	R	1
139	3.8	R	1	184	5.6	MR	3	229	19.1	MS	5
140	2.6	R	1	185	4.0	R	1	230	9.0	MR	3
141	12.6	MS	5	186	24.5	S	5	231	11.1	MR	3
142	8.3	MR	3	187	11.5	MR	3	232	8.3	MR	3
143	17.6	MS	5	188	11.1	MR	3	233	3.5	R	1
144	8.9	MR	3	189	6.9	MR	3	234	6.9	MR	3
145	3.6	R	1	190	9.8	MR	3	235	4.3	R	1
146	4.9	MR	3	191	10.8	MR	3	236	8.3	MR	3
147	9.0	MR	3	192	11.8	MR	3	237	10.9	MR	3
148	6.9	MR	3	193	9.8	MR	3	238	11.6	MR	3
149	3.3	R	1	194	11.3	MR	3	239	2.8	R	3
150	3.5	R	1	195	3.0	R	1	240	13.7	MS	5
151	2.7	R	1	196	12.7	MS	5	241	4.2	R	1
152	12.1	MS	5	197	12.4	MS	5	242	12.3	MS	5
153	12.6	MS	5	198	8.2	MR	3	243	6.9	MR	3
154	8.3	MR	3	199	14.2	MS	5	244	6.1	MR	3
155	5.7	MR	3	200	5.8	MR	3	245	3.1	R	1
156	4.9	R	1	201	4.5	R	1	246	6.7	MR	3
157	7.2	MR	3	202	2.6	R	1	247	4.7	R	1
158	10.6	MR	3	203	17.0	MS	5	248	7.1	MR	3
159	2.5	R	1	204	10.2	MR	3	249	6.8	MR	3
160	2.1	R	1	205	3.5	R	1	250	3.0	R	1
161	1.4	R	1	206	11.1	MR	3	251	4.7	R	1
162	10.3	MR	3	207	4.1	R	1	252	5.7	MR	3
163	4.1	R	1	208	3.1	R	1	253	2.4	R	1
164	18.7	MS	5	209	4.5	R	1	254	6.5	MR	3
165	7.4	MR	3	210	10.6	MR	3	255	7.6	MR	3
166	10.4	MR	3	211	11.4	MR	3	256	6.7	MR	3
167	8.9	MR	3	212	8.1	MR	3	257	9.0	MR	3
168	4.9	R	1	213	8.9	MR	3	258	3.6	R	1
169	3.1	R	1	214	18.7	MS	5	259	11.7	MR	3
170	17.5	MS	5	215	7.1	MR	3	260	2.4	R	1
171	11.7	MR	3	216	5.9	MR	3	261	11.8	MR	3
172	13.2	MS	5	217	9.0	MR	3	262	10.3	MS	5
173	5.0	R	1	218	8.6	MR	3	263	14.2	MS	5
174	11.8	MR	3	219	9.7	MR	3	264	3.1	R	1

F <sub>2</sub> Plant No.	% DI	Reaction	Score	F <sub>2</sub> Plant No.	% DI	Reaction	Score	F <sub>2</sub> Plant No.	% DI	Reaction	Score
265	15.6	MS	5	276	11.2	MR	3	287	24.5	MS	5
266	4.5	R	1	277	11.4	MR	3	288	36.8	S	7
267	5.3	MR	3	278	35.6	S	7	289	3.6	R	1
268	20.2	MS	5	279	2.5	R	1	290	11.2	MR	3
269	2.8	R	1	280	8.7	MR	3	291	2.4	R	1
270	4.4	R	1	281	2.7	R	1	292	12.8	MS	5
271	11.4	MS	5	282	5.0	R	1	293	12.7	MS	5
272	9.0	MR	3	283	9.8	MR	3	294	12.5	MS	5
273	10.5	MR	3	284	2.7	R	1	295	13.4	MS	5
274	11.9	MS	5	285	11.7	MR	3				
275	10.7	MR	3	286	10.8	MR	3				
<b>Parents and checks</b>											
TN1	77.3	HS	9	MTU 1010	50.9	HS	9				
YPB 46	32.7	S	7	IBT GM14	3.2	R	1				

%DI: percentage of disease incidence

susceptible (score 5) and susceptible (score 7). Many researchers screened segregating generations (F<sub>2</sub> and F<sub>3</sub>) of rice resistance against *Xoo* isolates of bacterial blight under field conditions using artificial leaf clip inoculation method described by Kauffman *et al.* (1973) and the scored by using IRRI, SES, 1996 and 2013.

Jamaloddin *et al.* (2020) screened selected nine ICF<sub>2</sub> plants, obtained from the cross TH/ISM//TH/NLR145, carrying BB resistance genes along with parents for bacterial blight resistance in field conditions using two most virulent isolates of *Xanthomonas oryzae* pv. *Oryzae* (DX-020 from Hyderabad, Telangana and IC31 from Maruteru, Andhra Pradesh), by following artificial leaf clip method of inoculation at maximum tillering stage at two different locations (Agricultural Research Institute (ARI), Hyderabad, India and Andhra Pradesh Rice Research Institute (APRRI), Maruteru, India). Scoring was done based on disease incidence percentage of IRRI, SES, 1996 and observed that all the nine plants carrying bacterial blight resistance genes were resistant to bacterial blight with a score 1.

Nine BC<sub>2</sub>F<sub>3</sub> plants pyramided with five bacterial blight resistance genes from the cross

IRBB66/TNG82), displayed a high level of resistance against the BB strain upon screening of seventeen BC<sub>2</sub>F<sub>3</sub> progenies for bacterial blight resistance at field conditions by inoculating the bacterial culture Taiwanese *Xanthomonas oryzae* strain isolate, XF89-b following leaf clip inoculation method (Hsu *et al.*, 2020). Nguyen *et al.* (2018) screened 11 BC<sub>3</sub>F<sub>3</sub> plants from the cross LT2 and IRBB21 for bacterial blight under field conditions by following artificial leaf clip inoculation (Kaufmann *et al.*, 1973). Scoring of the plants was done at 18 DAI according to SES (IRRI, 1996) and observed that all the plants were resistant to bacterial blight.

Arunakumari *et al.* (2016) used a virulent *Xoo* isolate, DX-022 to screen the donor and recurrent parents (ISM and NLR145) along with ICF<sub>2</sub> plants from the cross ISM and NLR145 for BB resistance following leaf clip method of inoculation and disease score was evaluated as per SES (IRRI, 1996). The donor genotype ISM showed an average lesion length of 0.77 cm with disease score 1, while the recurrent parent MTU1010 possessed an average lesion length of 12.23 cm (90% diseased leaf area) with disease score of 9. All the three-gene pyramid ICF<sub>2</sub> plants showed highly resistant reaction against the disease with a lesion length of only 0.53–2.28 cm and 1–5% diseased leaf area with disease scoring values of 1 or 3.



## CONCLUSION

The 230 resistant plants (88 resistant and 142 moderately resistant) identified after screening of 295  $F_2$  plants for bacterial blight resistance are valuable and these plants could be evaluated further till homozygosity is attained. The plants which are resistant to bacterial blight and high yield would be selected towards development of high yielding resistant varieties.

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## INFLUENCE OF DIFFERENT NITROGEN LEVELS ON GROWTH AND YIELD OF DIFFERENT RICE CULTIVARS

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### ABSTRACT

A field experiment was conducted during three consecutive seasons (Kharif 2020, Rabi 2021 and Kharif 2021) at IIRR farm, Rajendranagar, Hyderabad to study the effect of different nitrogen levels application on growth and development in rice. The experiment was laid out in split plot design with four nitrogen levels as main plots, fourteen varieties as sub plots and replicated thrice. Among the nitrogen treatments, application of 150 kg N ha<sup>-1</sup> recorded highest plant height (103.5, 98.1 and 98.7 cm), number of tillers hill<sup>-1</sup> (9.5, 10.0 and 7.9), LAI (5.29, 5.06 and 5.20), SPAD value (45.3, 44.4 and 43.6), grain yield (5.16, 5.22 and 4.94 t/ha) and more number of days to panicle initiation (78, 79 and 77 days), 50% anthesis (100, 103 and 98 days) and maturity (130, 130 and 128 days) whereas lowest plant height (86.1, 82.2 and 86.1 cm), number of tillers hill<sup>-1</sup> (5.7, 6.6 and 5.7), LAI (2.41, 2.40 and 2.49), SPAD value (38.3, 37.6 and 36.4), grain yield (2.76, 2.89 and 2.78 t/ha) and less number of days to panicle initiation (76, 77 and 74 days), 50% anthesis (96, 98 and 93 days) and maturity (125, 125 and 125 days) was noticed in 0 kg N ha<sup>-1</sup> in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Anjali and Heerarecorded mean minimum number of days for panicle initiation, 50% anthesis and maturity whereas mean maximum number of days for panicle initiation, 50% anthesis and maturity were taken by Birupa and Daya. MTU-1010 recorded highest mean grain yield (4.83, 4.78 and 4.78 t/ha) whereas lowest grain yield was noticed in N-22 (3.01, 2.90 and 2.40 t/ha) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively.

**KEY WORDS:** Nitrogen, rice, leaf area index, grain yield.

Rice is one of the most important cereal crops of the world and contributes half of the world's staple food (Sporchia *et al.*, 2021). Rice is grown in a wide range of climatic conditions covering one-third of the world's total-cropped area. In India, rice is grown in an area of 44.5 M ha with a production 115.60 Mt and a productivity of 2800 kg ha<sup>-1</sup>. Telangana State contributes 2.09 M ha area annually with a production of 6.62 Mt, with an average productivity of 3295 kg ha<sup>-1</sup> during 2018-2019 (CMIE, 2019).

Among the major nutrient elements, nitrogen (N) is the most limiting nutrient for rice crop growth and yield which is required in higher amounts compared to other nutrients (Djaman *et al.*, 2018). N influences rice yield by playing major role in the photosynthesis, biomass accumulation, effective tillering, and spikelets formation (Yoshida *et al.*, 2006). Nitrogen is essential nutrient element for rice growth and metabolic processes (Noor, 2017; Ghoneim and Ebid, 2015). Nitrogen absorbed by rice during the vegetative growth stages contributes in

growth during reproduction and grain filling through translocation. The application of nitrogen fertilizer either in excess or less than optimum rate affects both yield and quality of rice to remarkable extent, hence proper management of crop nutrition is of immense importance (Manzoor *et al.*, 2006). Efficient use of N chemical fertilizers can be attained through cultural and agronomic practices. Most importantly by breeding varieties having maximum NUE, thereby reducing risks of environmental and soil water pollution with low nitrogen inputs (Noor, 2017; Fageria *et al.*, 2008; Sachiko *et al.*, 2009).

Managing nitrogen fertilization is a challenging task for farmers in rice fields because of various losses due to de-nitrification, volatilization, leaching in flooded soils resulting in low uptake and poor nitrogen use efficiency (Peng *et al.*, 2006). Limited application of N fertilizer causes N deficiency in rice plants which increases yellowing in colour and reduction in leaf size. Reduced N supply at tillering and panicle initiation stages ultimately lead to a reduction in grain yield

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(Hussain *et al.*, 2022). Excess application leads to lodging, pest and disease incidence whereas low application results in limited growth and reduced yields. The higher dose of nitrogen causes excessive vegetative growth that leads to lodging of the crop and a consequent decline in filled grains per panicle (Zhang *et al.*, 2014). Rice genotypes differ in their efficiency in utilizing the soil available N and also in their response to added N which can be explored and further utilized in the development of efficient genotypes for N limiting environments. Therefore, the present study was conducted to evaluate the influence of different levels of nitrogen application on growth and grain yield of different rice cultivars.

### MATERIAL AND METHODS

Field experiment was conducted on clayey vertisol at IIRR farm, Rajendranagar, Hyderabad during Kharif 2020, Rabi 2021 and Kharif 2021. The experiment was laid out in a split plot design with three replications. Nitrogen levels were given as 0% RDN (0 Kg N ha<sup>-1</sup>), 50% RDN (50 Kg N ha<sup>-1</sup>), 100% RDN (100 Kg N ha<sup>-1</sup>) and 150% RDN (150 Kg N ha<sup>-1</sup>) taken as main plots. The seedlings of different rice varieties V1- Anjali, V2 -Birupa, V3 -Daya, V4 - GQ-25, V5 -Heera, V6 - Indira, V7 - IR-64, V8 - MTU-1010, V9 - N-22, V10 -Nidhi, V11 - TellaHamsa, V12 - V L Dhan, V13 - Varadhan and V14 - Vasumathi were selected as sub plots. The varieties were sown separately in raised bed nursery (Kharif-2020 – 20-06-2020; Rabi-2021 – 02-01-2021; Kharif-2021 – 18-06-2020) and twenty five day old seedlings were transplanted into main field by adopting a spacing of 20 cm between rows and 10 cm within a row. Nitrogen applied as per treatment in form of urea in 3 splits at basal, maximum tillering and flowering stage. Phosphorus was applied as single super phosphate at the rate of 60 kg ha<sup>-1</sup> and Potash as muriate of potash at the rate of 40 kg ha<sup>-1</sup> as a basal dose at the time of transplanting. Irrigation and weed management was done time to time. Prophylactic measures were taken to prevent damage due to pests and diseases.

Plant height was measured from the base of the stem to the topmost leaf or panicle at 50% anthesis. The number of tillers hill<sup>-1</sup> was counted in net plot area and expressed as tillers hill<sup>-1</sup>. The number of days taken to panicle initiation, 50 % flowering and maturity from

sowing in each variety in each plot were recorded. The SPAD (Soil Plant Analytical Development) chlorophyll meter readings were measured with SPAD 502; Minolta Company Ltd which measures the greenness or relative chlorophyll content of leaves. The third leaf from top was used for measuring SCMR, which was taken midway between the leaf base and tip. Leaf area was measured at 50% anthesis by using leaf area meter and LAI was calculated as per Watson (1952).

$$\text{LAI} = \frac{\text{Total leaf area of a plant}}{\text{Ground area covered by plant}}$$

The crop was harvested manually and grain yield per net plot was recorded and converted to grain yield ha<sup>-1</sup>.

The different data were subjected to pooled analysis of variance (ANOVA) as split-plot design using SAS 9.0. Graphics were constructed using R software. Mean values were calculated and significance of the difference between treatments was tested by LSD (least significant difference) method at the significance level of P=0.01 or 0.05.

### RESULTS AND DISCUSSION

#### Plant Height (cm)

In the present study, the different nitrogen treatments significantly influenced the rice growth parameters. Significant increase in plant height was noticed with increasing N application. The data on plant height recorded at 50% anthesis (Table 1) indicated that significantly taller plants (103.5, 98.1 and 98.7 cm) were observed with 150 kg N ha<sup>-1</sup> while shorter plants (86.1, 82.2 and 86.1 cm) were observed at 0 kg N ha<sup>-1</sup> in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant differences were observed among the tested varieties for plant height. Significant interaction was observed for plant height between varieties and seasons. Among the varieties mean higher plant height was noticed in Varadhan during kharif-2020 (107.3), Indira and V L Dhan during rabi-2021 (99.9) and Vasumathi during kharif-2021 (108.1) whereas mean lowest was recorded in MTU-1010 during kharif-2020 (82.6), and Daya during rabi-2021 (82.7) and Kharif-2021 (77.6). Significant interaction was observed between the treatments and varieties for plant height. An increase in plant height

**Table 1. Influence of Nitrogen levels on plant height in different rice cultivars. Each value represents the mean of 3 replications.**

Varieties	Plant height (cm)														
	Kharif-2020					Rabi-2021					Kharif-2021				
	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean
Anjali	89.3	98.3	110.3	119.3	104.3	84.8	91.2	98.8	102.0	94.2	88.7	93.5	100.0	104.7	96.7
Birupa	81.8	92.8	99.2	102.7	94.1	77.7	85.2	92.8	99.8	88.9	89.8	93.8	97.5	101.2	95.6
Daya	84.2	86.0	92.7	93.0	89.0	73.3	79.3	87.5	90.5	82.7	71.2	74.8	79.3	85.0	77.6
GQ-25	81.5	89.8	94.8	97.3	90.9	84.0	87.5	91.3	94.2	89.3	83.8	85.5	90.2	93.8	88.3
Heera	74.7	83.7	90.3	95.5	86.0	79.0	82.7	87.2	91.2	85.0	80.7	85.0	88.2	90.7	86.1
Indira	92.7	98.8	103.5	107.5	100.6	90.0	98.8	104.5	106.2	99.9	77.2	82.7	90.3	101.7	88.0
IR-64	82.0	88.2	91.8	92.2	88.5	77.2	83.5	87.8	90.7	84.8	75.3	78.5	81.0	82.3	79.3
MTU-1010	79.7	81.2	84.3	85.2	82.6	80.8	83.3	85.2	85.3	83.7	78.3	80.0	83.2	86.7	82.0
N-22	82.2	96.3	110.5	116.2	101.3	81.8	86.8	94.5	99.8	90.8	96.3	99.7	102.7	108.7	101.8
Nidhi	93.2	98.7	102.3	103.2	99.3	79.5	87.2	94.8	97.8	89.8	94.8	95.5	98.3	101.2	97.5
TellaHamsa	73.3	86.5	92.7	95.7	87.0	81.5	89.8	93.2	95.5	90.0	89.0	93.7	96.3	99.0	94.5
V L Dhan	99.3	103.7	107.7	110.8	105.4	90.3	96.5	104.5	108.2	99.9	95.0	98.5	105.3	107.7	101.6
Varadhan	93.8	102.5	114.2	118.7	107.3	86.2	95.5	106.2	110.2	99.5	83.3	94.3	101.5	103.7	95.7
Vasumathi	97.2	100.8	106.3	112.0	104.1	84.7	92.7	98.5	102.5	94.6	102.2	105.3	109.5	115.3	108.1
Mean	86.1	93.4	100.0	103.5	95.8	82.2	88.6	94.8	98.1	90.9	86.1	90.1	94.5	98.7	92.3
LSD Treatment (T)	2.17**														
LSD Variety (V)	2.87**														
LSD Season (S)	NS														
LSD (T x V)	5.75**														
LSD (T x S)	NS														
LSD (V x S)	4.98**														
LSD (T x V x S)	NS														
CV (%)	5.06														

\*p<0.05, \*\*p<0.001

occurred possibly due to the contribution of added N which improved the growth, internode length and overall metabolism. Enhanced N application is well documented in encouraging cell expansion, and it subsequently stimulated stem elongation (Wu *et al.*, 2020). Results for plant height were supported by the findings of Abbasi *et al.* (2012) and Zhang *et al.* (2020) who reported remarkable improvements in plant height following increased N application rate. Similar results have also been demonstrated by Jahan *et al.*, (2020) who described that an increase in N supply to rice genotypes caused a significant increase in the height of rice plants.

#### Number of tillers hill<sup>-1</sup>

Number of tillers per unit area is the most important component of yield. More the number of tillers, especially fertile tillers; the more will be the yield. Data on number of tillers hill<sup>-1</sup> at maturity is presented in Table 2. Significant increase in number of tillers hill<sup>-1</sup> was observed with increasing N application. Number of tillers hill<sup>-1</sup> has increased with increase in nitrogen application. Application of nitrogen at 150 kg N ha<sup>-1</sup> has resulted in mean maximum number of tillers hill<sup>-1</sup> (9.5, 10.0 and 7.9), while at 0 kg N ha<sup>-1</sup> mean minimum number of tillers hill<sup>-1</sup> (5.7, 6.6 and 5.7) were recorded in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. The tested varieties differed significantly for number of tillers hill<sup>-1</sup>. Significant interaction was observed for number of tillers hill<sup>-1</sup> between varieties and seasons. Among the varieties, the mean higher number of tillers hill<sup>-1</sup> were observed in N-22 during all three tested seasons while mean lowest number of tillers hill<sup>-1</sup> were noted in Birupa (5.6) during kharif-2020, MTU-1010 (6.9) during rabi-2021 and Vasumathi (5.5) during kharif-2021. Interaction between N treatments and varieties was found to be significant for number of tillers hill<sup>-1</sup>. Wang *et al.* (2018) demonstrated that N availability controls rice tiller numbers through the regulation of the nitrate transporter. An elevated nitrogen level in rice plants leads to increased tiller numbers and tiller bud outgrowth (Chen *et al.*, 2020). Jahan *et al.* (2020) observed that N fertilization increased the number of tillers m<sup>2</sup>, which resulted due to the increased N availability for cell division.

#### Days to panicle initiation

Panicle initiation is the time when the panicle primordia initiate the production of a panicle in the

uppermost node of the culm. Significant differences were observed between the treatments and among the varieties for days to panicle initiation (Table 3). In all three seasons, mean maximum number of days to panicle initiation was recorded in treatment supplied with 150 kg N ha<sup>-1</sup> (78, 79 and 77 days), while mean minimum in treatment of 0 kg N ha<sup>-1</sup> (76, 77 and 74 days) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant interaction was observed for days to panicle initiation between varieties and seasons. Among the varieties under study, mean minimum number of days for panicle initiation was recorded in the varieties Anjali and Heera (64 days) during kharif-2020, Heera (65 days) during rabi-2021 and Anjali (64 days) during kharif-2021, while the mean maximum number of days to panicle initiation was taken by Birupa and Daya (87 days) during kharif-2020, Daya (90 days) during rabi-2021 and Birupa (86 days) during kharif-2021. Significant interaction was observed between N treatments and varieties for days to panicle initiation. With increase in dose of applied nitrogen, the number of days to panicle initiation has increased. Abou-Khalifa (2007) found that days to maximum tillering, panicle initiation, and heading increased with increased levels of nitrogen up to 165 kg N ha<sup>-1</sup>.

#### Days to 50% anthesis

Anthesis refers to the events between the opening and closing of the spikelet. Significant differences were recorded in the number of days taken for 50 % anthesis between the treatments and among the varieties (Table 4). Timing of anthesis was delayed with higher dose of nitrogen. Among various nitrogen levels, data revealed that application of 150 kg N ha<sup>-1</sup> taken mean maximum number of days (100, 103 and 98 days) to anthesis, whereas treatment of 0 kg N ha<sup>-1</sup> flowered early (96, 98 and 93 days) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant interaction was observed for days to 50% anthesis between varieties and seasons. Anjali and Heera (82 days) during kharif-2020, Heera (86 days) during rabi-2021 and Anjali (80 days) during kharif-2021 recorded mean minimum number of days for 50 % anthesis, while the mean maximum was observed in Birupa (111 days) during kharif-2020, Birupa and Daya (114 days) during rabi-2021, and Birupa (108 days) during kharif-2021. Interaction between nitrogen levels and varieties was

**Table 2. Influence of Nitrogen levels on number of tillers in different rice cultivars. Each value represents the mean of 3 replications.**

Varieties	Number of tillers hill <sup>-1</sup>																	
	Kharif-2020						Rabi-2021						Kharif-2021					
	N0	N50	N100	N150	Mean		N0	N50	N100	N150	Mean		N0	N50	N100	N150	Mean	
Anjali	5.2	6.7	7.3	7.7	6.7		6.0	7.7	8.5	9.3	7.9		6.5	6.7	6.8	7.0	6.8	
Birupa	4.0	5.3	6.3	6.8	5.6		5.2	6.8	8.2	8.7	7.2		5.7	5.7	6.0	6.5	6.0	
Daya	6.5	7.5	9.8	10.3	8.5		6.5	8.2	10.2	11.3	9.0		5.5	6.7	7.3	9.7	7.3	
GQ-25	6.0	9.0	10.0	11.7	9.2		7.2	8.3	9.2	10.3	8.8		5.0	5.8	7.0	7.2	6.3	
Heera	5.0	7.0	9.0	10.5	7.9		6.3	8.2	8.8	10.2	8.4		5.3	6.5	7.2	7.8	6.7	
Indira	4.3	5.8	6.5	7.7	6.1		6.0	6.5	7.7	8.7	7.2		5.5	7.7	8.7	9.0	7.7	
IR-64	5.8	8.2	10.0	10.7	8.7		6.5	7.8	9.7	9.7	8.4		5.5	6.0	7.5	8.3	6.8	
MTU-1010	5.7	6.0	7.3	7.7	6.7		5.8	6.3	7.5	7.8	6.9		5.2	5.7	6.8	7.3	6.3	
N-22	9.0	11.3	11.7	11.8	11.0		9.0	10.7	11.3	11.7	10.7		7.0	8.0	9.3	9.3	8.4	
Nidhi	6.2	7.3	8.2	8.8	7.6		7.2	8.3	9.0	10.0	8.6		6.3	6.8	8.0	8.7	7.5	
TellaHamsa	5.2	8.8	10.8	11.3	9.0		6.8	8.5	9.2	10.3	8.7		6.3	6.8	7.7	7.8	7.2	
V L Dhan	5.8	7.7	8.5	8.8	7.7		7.0	7.7	9.8	10.5	8.8		5.5	6.0	6.7	6.8	6.3	
Varadhan	5.5	7.7	9.3	9.8	8.1		7.0	9.0	10.2	11.2	9.3		6.2	7.3	9.2	9.2	8.0	
Vasumathi	5.5	7.0	8.2	8.7	7.3		6.0	7.3	9.7	10.3	8.3		4.3	5.5	6.0	6.3	5.5	
Mean	5.7	7.5	8.8	9.5	7.9		6.6	8.0	9.2	10.0	8.4		5.7	6.5	7.4	7.9	6.9	
LSD Treatment (T)	0.266**																	
LSD Variety (V)	0.626**																	
LSD Season (S)	NS																	
LSD (T x V)	0.951*																	
LSD (T x S)	NS																	
LSD (V x S)	1.08**																	
LSD (T x V x S)	NS																	
CV (%)	13.27																	

\*p<0.05, \*\*p<0.001

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Table 3. Influence of Nitrogen levels on days to panicle initiation in different rice cultivars. Each value represents the mean of 3 replications.

Varieties	Days to panicle initiation														
	Kharif-2020					Rabi-2021					Kharif-2021				
	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean
Anjali	63	64	65	65	64	68	68	68	69	68	63	64	64	65	64
Birupa	88	88	87	87	87	87	88	88	89	88	85	86	86	87	86
Daya	86	87	87	87	87	89	89	90	91	90	83	84	85	86	85
GQ-25	81	83	83	85	83	79	79	79	80	79	76	77	78	79	78
Heera	63	64	64	64	64	64	65	66	66	65	64	64	65	66	65
Indira	81	82	83	83	83	82	83	84	84	84	80	81	82	84	82
IR-64	82	82	83	84	83	77	78	78	78	78	77	78	79	80	79
MTU-1010	72	72	72	72	72	71	71	71	70	71	71	72	73	74	72
N-22	68	68	68	68	68	70	71	72	73	72	68	69	70	71	70
Nidhi	82	84	85	85	84	76	78	78	78	78	80	82	83	83	82
TellaHamsa	65	66	66	66	66	71	73	73	74	73	64	65	66	66	65
V L Dhan	82	83	83	84	83	75	77	77	78	77	79	80	81	82	80
Varadhan	73	74	74	74	74	78	79	80	80	79	69	70	71	72	71
Vasumathi	85	85	85	84	85	86	87	88	89	88	83	85	86	87	85
Mean	76	77	77	78	77	77	78	78	79	78	74	76	76	77	76
LSD Treatment (T)	0.352**														
LSD Variety (V)	0.774**														
LSD Season (S)	NS														
LSD (T x V)	1.176*														
LSD (T x S)	NS														
LSD (V x S)	1.34**														
LSD (T x V x S)	NS														
CV (%)	1.64														

\*p<0.05, \*\*p<0.001

**Table 4. Influence of Nitrogen levels on days to 50% anthesis in different rice cultivars. Each value represents the mean of 3 replications**  
Days to 50% anthesis

Varieties	Kharif-2020					Rabi-2021					Kharif-2021				
	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean
Anjali	79	81	83	84	82	86	87	88	90	88	78	80	81	82	80
Birupa	110	111	111	112	111	111	113	114	116	114	106	108	109	110	108
Daya	107	109	110	111	109	112	113	115	117	114	103	105	107	108	106
GQ-25	101	104	105	108	104	101	102	103	105	103	95	97	99	100	98
Heera	80	82	83	84	82	83	85	87	88	86	80	81	83	84	82
Indira	102	104	106	107	105	105	107	109	110	108	99	102	104	105	103
IR-64	101	103	105	107	104	99	101	102	103	101	96	98	100	101	99
MTU-1010	92	92	93	94	93	93	94	94	95	94	89	91	93	94	92
N-22	86	87	88	89	88	90	92	94	96	93	85	87	89	90	88
Nidhi	102	105	107	108	105	98	101	102	103	101	99	102	104	104	102
TellaHamsa	82	84	85	86	84	90	93	94	96	93	80	82	84	84	83
V L Dhan	102	104	105	107	104	97	100	101	103	100	98	100	102	103	101
Varadhan	92	94	95	96	94	99	101	103	104	102	87	89	91	92	90
Vasumathi	106	107	108	108	107	109	111	113	115	112	103	106	108	109	106
Mean	96	98	99	100	98	98	100	101	103	101	93	95	97	98	96
LSD Treatment (T)	0.352**														
LSD Variety (V)	0.598**														
LSD Season (S)	NS														
LSD (T x V)	0.909*														
LSD (T x S)	NS														
LSD (V x S)	1.03**														
LSD (T x V x S)	NS														
CV (%)	0.999														

\*p<0.05, \*\*p<0.001



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found to be significant for 50 % anthesis. Abundant supply of nitrogen ( $150 \text{ kg N ha}^{-1}$ ), which is higher than RDN ( $100 \text{ kg N ha}^{-1}$ ) might have delayed the vegetative growth and shifted the balance between vegetative and reproductive growth, leading to delay in days to 50 % heading (Venugopal, 2005).

### Days to maturity

This stage corresponds to the complete grain filling where grain increases in size and weight as the starch and sugars are translocated from the culms and leaf sheaths and the whole grain is hard and ready for harvest (Nagesha *et al.*, 2019). Data pertaining to days taken for maturity has been presented in table 5. There was significant difference between treatments and among the varieties for number of days taken for maturity. Among different levels of applied nitrogen,  $150 \text{ kg N ha}^{-1}$  has taken mean maximum number of days (130, 130 and 128 days) to maturity whereas treatment of  $0 \text{ kg N ha}^{-1}$  has recorded mean minimum number of days (125, 125 and 125 days) to grain maturity in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant interaction was observed for days to maturity between varieties and seasons. Birupa (140 days) during kharif-2020, Daya (136 days) during rabi-2021 and Birupa (137 days) during kharif-2021 has showed mean maximum number of days for maturity, while mean minimum being taken by Anjali (111 days) during kharif-2020, Heera (117 days) during rabi-2021, and Anjali and TellaHamsa (112 days) during kharif-2021. Significant interaction was observed between nitrogen levels and varieties for days to maturity. Wani *et al.*, (2017) reported that delayed flowering with higher nitrogen dose may be due to more vegetative growth, as reflected by increased plant height, which delayed maturity.

### Leaf area index (LAI)

The ultimate factors which limit the primary process in crop production i.e. crop photosynthesis is the efficiency of light captured and utilization, which depends on leaf area. Leaf area index (LAI) at 50% anthesis has been depicted in figure 1. Significant increase in LAI was observed with increasing N application. The data clearly pinpoints the fact that the leaf area index increased with increasing doses of nitrogen. Highest mean LAI was recorded in treatment supplied with  $150 \text{ kg N ha}^{-1}$  (5.29, 5.06 and 5.20), whereas mean minimum LAI was recorded in treatment

of  $0 \text{ kg N ha}^{-1}$  (2.41, 2.40 and 2.49) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant differences were noticed among the tested varieties for LAI. Significant interaction was observed for LAI between varieties and seasons. GQ-25 (4.48) during kharif-2020, Varadhan (4.37) during rabi-2021 and Birupa (4.58) during kharif-2021 has recorded higher mean LAI whereas least mean LAI was observed in Anjali during kharif-2020 (3.33) and rabi-2021 (3.46) and, IR-64 during kharif-2021 (3.26). Interaction effect was found to be significant between treatments and varieties for LAI. The increasing trend of LAI at higher nitrogen levels can be attributed to the positive effect of nitrogen on both leaf development and leaf area duration of the variety (Fageria, 2007; Fageria and Baligar, 2005). Progressive increment in LAI of the variety with increase in N application may be due to the fact that addition of nitrogen triggers increased number of leaves per plant and expansion of individual leaf (Haque and Haque, 2016).

### Chlorophyll content

Greenness or relative chlorophyll content of leaves was measured with SPAD. Results on SCMR as influenced by nitrogen application in rice genotypes were presented in figure 2. There was significant increase in SCMR values with increasing nitrogen application. Among the treatments, application of  $150 \text{ kg N ha}^{-1}$  resulted in higher mean SCMR values (45.3, 44.4 and 43.6), whereas plants which were grown in  $0 \text{ kg N ha}^{-1}$  recorded the lower mean SCMR values (38.3, 37.6 and 36.4) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. The tested varieties differed significantly for SCMR values. Significant interaction was observed for SCMR values between varieties and seasons. Among the varieties N-22 and Vasumathi during kharif-2020 (40.8), V L Dhan during rabi-2021 (38.6) and N-22 during kharif-2021 (35.7) has recorded least mean SCMR values whereas Heera during kharif-2020 (43.8), MTU-1010 and Vasumathi during rabi-2021 (43.0), and Anjali and TellaHamsa during kharif-2021 (43.9) has recorded higher mean SCMR values. Significant interaction was observed between N treatments and varieties for SCMR values. The linear relationships for chlorophyll content and N application rate have been documented by Abunyewa *et al.*, (2016).

**Table 5. Influence of Nitrogen levels on days to maturity in different rice cultivars. Each value represents the mean of 3 replications.**

Varieties	Days to maturity														
	Kharif-2020					Rabi-2021					Kharif-2021				
	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean
Anjali	110	111	112	113	111	116	119	116	119	118	110	111	113	113	112
Birupa	137	139	140	142	140	132	133	135	137	134	135	137	138	139	137
Daya	135	139	140	142	139	133	135	136	138	136	135	135	136	137	136
GQ-25	130	134	135	136	134	130	131	133	135	132	124	126	127	128	126
Heera	113	114	114	115	114	114	115	118	119	117	117	118	119	120	119
Indira	130	133	137	138	134	129	130	132	134	131	126	127	128	129	128
IR-64	129	134	135	136	134	127	130	132	133	131	132	133	134	135	133
MTU-1010	120	123	123	125	123	122	124	124	125	124	124	125	127	128	126
N-22	117	118	119	120	119	118	120	123	125	122	118	119	121	122	120
Nidhi	126	132	135	138	133	126	129	130	131	129	129	130	131	132	131
TellaHamsa	115	117	118	119	117	119	121	123	126	122	110	112	113	114	112
V L Dhan	133	136	136	138	136	125	127	130	131	128	127	128	129	130	129
Varadhan	123	125	126	127	125	128	130	133	134	131	122	123	124	126	124
Vasumathi	133	136	138	138	136	131	132	134	135	133	134	135	137	138	136
Mean	125	128	129	130	128	125	127	129	130	128	125	126	127	128	126
LSD Treatment (T)	0.492**														
LSD Variety (V)	0.663**														
LSD Season (S)	NS														
LSD (T x V)	1.006*														
LSD (T x S)	NS														
LSD (V x S)	1.14**														
LSD (T x V x S)	NS														
CV (%)	0.852														

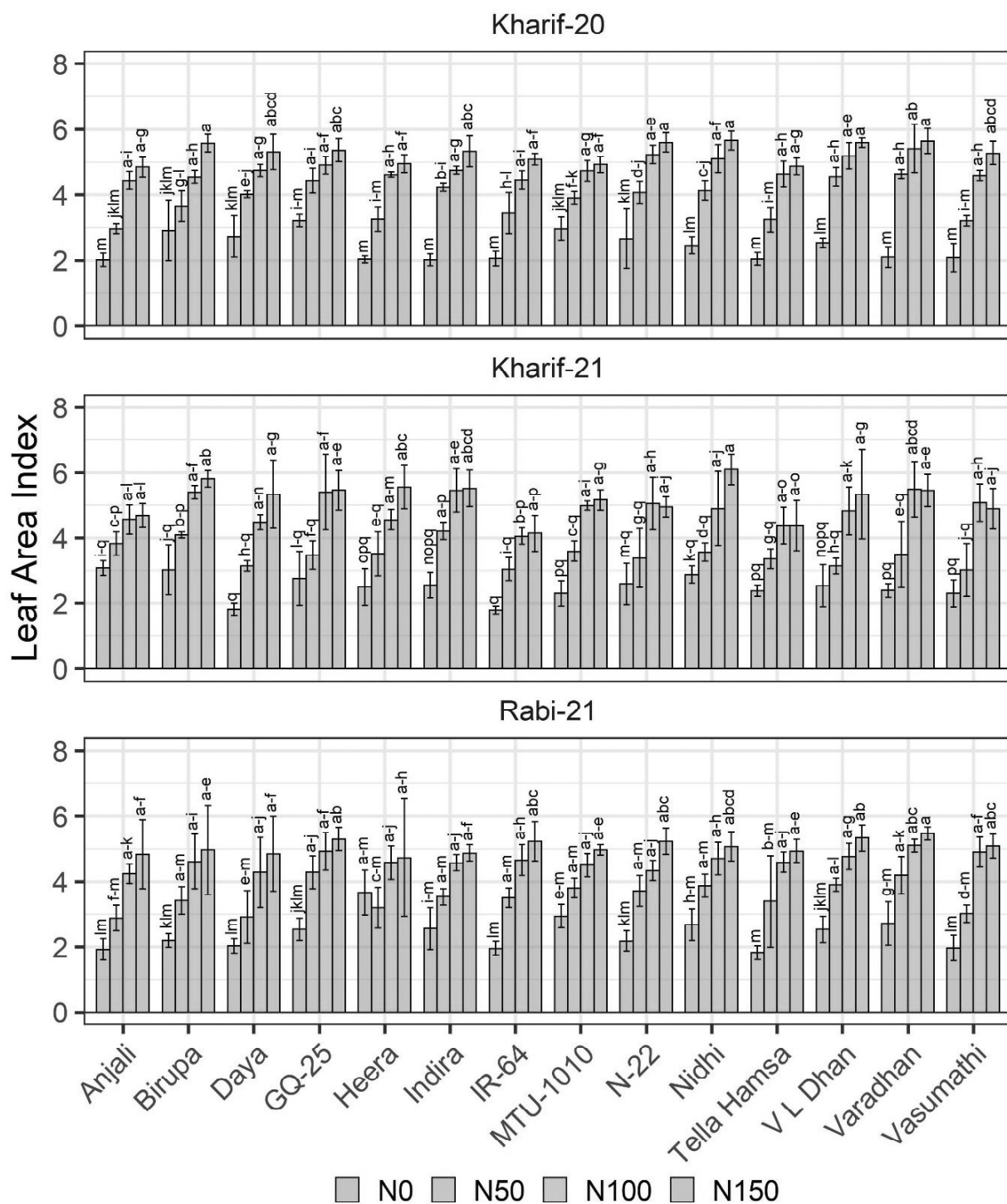
\*p<0.05, \*\*p<0.001

INFLUENCE OF DIFFERENT NITROGEN LEVELS ON GROWTH

Grain yield

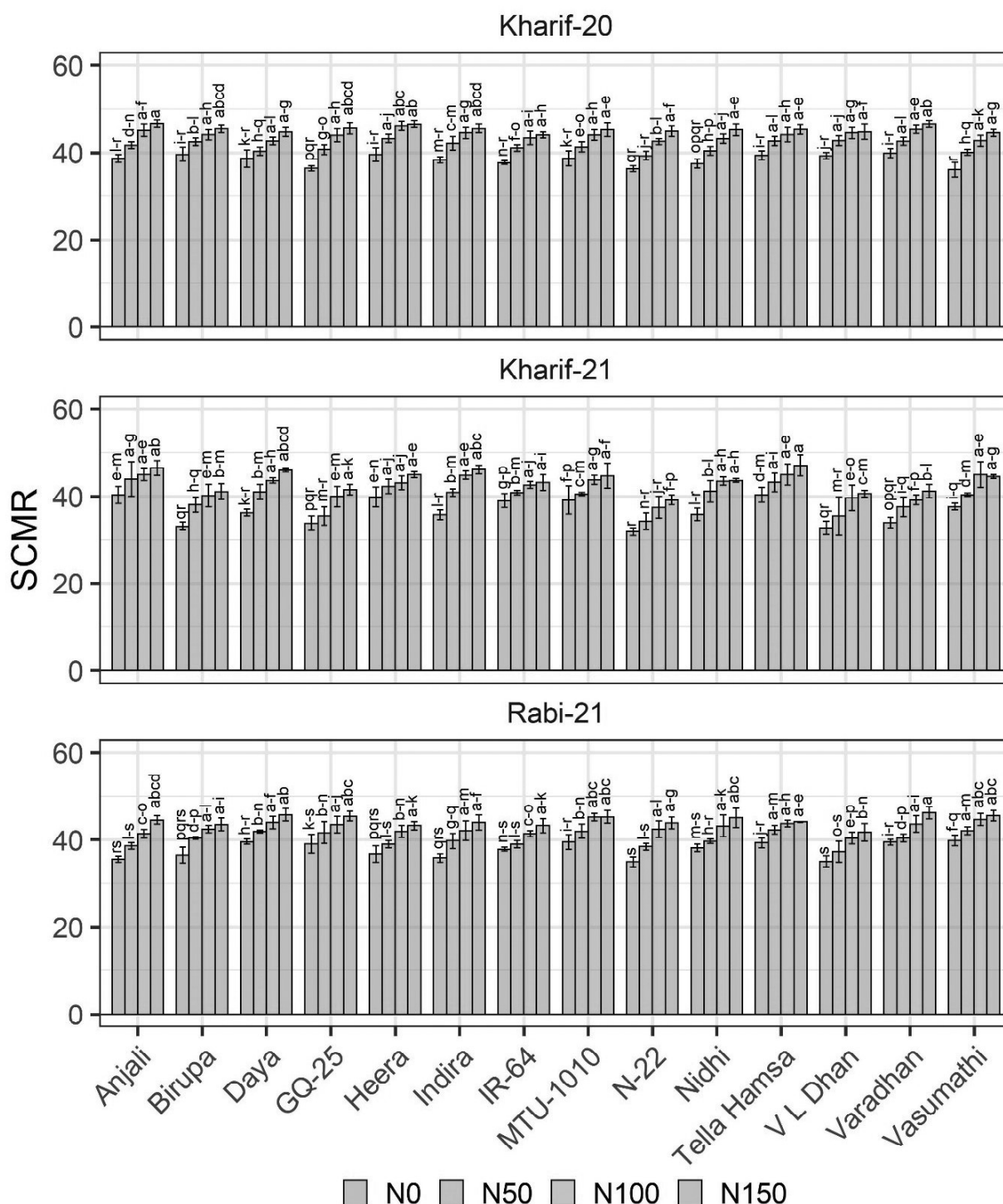
Grain yield is a complex heritable character influenced by many morphological, physiological and biochemical characteristics of the plant interacting with the environment. A perusal of the data presented

in table 6 on grain yield indicates that with increase in nitrogen application there was significant increase in the grain yield. Results showed that mean maximum grain yield has been recorded in treatment supplied with 150 kg N ha<sup>-1</sup> (5.16, 5.22 and 4.94 t/ha), whereas



\*Treatments with same letters are not significantly different.

Figure 1. Influence of Nitrogen levels on leaf area index in different rice cultivars. Each bar represents the mean of 3 replications



\*Treatments with same letters are not significantly different.

**Figure 2. Influence of Nitrogen levels on SCMR in different rice cultivars. Each bar represents the mean of 3 replications**

mean lowest grain yield was observed in treatment of 0 kg N ha<sup>-1</sup> (2.76, 2.89 and 2.78 t/ha) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Significant differences were noticed among the varieties also for grain yield. Significant interaction was observed for grain yield between varieties and seasons. Among the varieties MTU-1010 recorded mean highest grain yield

(4.83, 4.78 and 4.78 t/ha) whereas lowest mean grain yield was noticed in N-22 (3.01, 2.90 and 2.40 t/ha) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Interaction effect between nitrogen treatment and varieties for grain yield was found to be significant. N-22 with 0 kg N ha<sup>-1</sup> recorded least grain yield (1.94, 2.05 and 1.65 t/ha) whereas MTU-1010 with 150 kg

**Table 6. Influence of Nitrogen levels on grain yield in different rice cultivars. Each value represents the mean of 3 replications.**

Varieties	Grain yield (t/ha)														
	Kharif-2020					Rabi-2021					Kharif-2021				
	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean	N0	N50	N100	N150	Mean
Anjali	2.55	3.23	4.68	5.01	3.87	2.70	3.38	5.19	5.40	4.17	2.34	2.91	4.30	4.53	3.52
Birupa	3.00	4.11	4.89	5.24	4.31	2.94	4.15	4.95	5.11	4.29	2.77	3.96	5.11	4.59	4.11
Daya	2.71	3.64	5.20	5.12	4.17	2.73	3.69	5.37	5.43	4.31	2.71	3.14	5.17	5.35	4.09
GQ-25	2.77	3.71	5.05	5.32	4.21	2.66	4.00	5.34	5.44	4.36	2.44	3.64	4.57	4.83	3.87
Heera	3.03	3.74	5.02	5.09	4.22	3.39	4.03	5.02	5.07	4.38	3.59	3.94	4.83	5.33	4.42
Indira	2.90	3.90	5.58	5.69	4.52	2.94	4.17	5.80	5.86	4.69	3.08	4.02	5.93	5.81	4.71
IR-64	3.10	3.58	4.95	5.13	4.19	3.35	3.87	5.33	5.42	4.49	3.61	3.73	5.85	5.00	4.55
MTU-1010	3.46	4.14	5.84	5.87	4.83	3.36	4.10	5.81	5.84	4.78	3.45	4.11	5.71	5.86	4.78
N-22	1.94	2.43	3.70	3.96	3.01	2.05	2.67	3.25	3.65	2.90	1.65	2.27	2.80	2.88	2.40
Nidhi	2.39	3.59	4.70	4.91	3.90	2.54	3.46	5.03	5.00	4.01	2.23	3.18	4.00	4.83	3.56
TellaHamsa	2.19	2.99	4.14	4.29	3.40	2.42	3.13	3.83	3.97	3.34	2.26	2.74	3.38	3.22	2.90
V L Dhan	3.23	3.81	5.45	5.39	4.47	3.48	3.81	5.20	5.44	4.48	2.55	4.01	5.77	5.78	4.53
Varadhan	2.56	4.16	5.57	5.60	4.47	3.10	4.42	5.59	5.78	4.72	2.84	4.18	5.71	5.27	4.50
Vasumathi	2.88	4.33	5.59	5.62	4.61	2.83	4.15	5.47	5.62	4.52	3.38	3.48	5.04	5.80	4.43
Mean	2.76	3.67	5.03	5.16	4.16	2.89	3.79	5.08	5.22	4.24	2.78	3.52	4.87	4.94	4.03
LSD Treatment (T)	0.106**														
LSD Variety (V)	0.194**														
LSD Season (S)	NS														
LSD (T x V)	0.388**														
LSD (T x S)	NS														
LSD (V x S)	0.336**														
LSD (T x V x S)	NS														
CV (%)	7.67														

\*p<0.05, \*\*p<0.001

N ha<sup>-1</sup> recorded highest grain yield (5.87, 5.84 and 5.84 t/ha) in Kharif-2020, Rabi-2021 and Kharif-2021, respectively. Incremental increase of nitrogen recorded significant increase in grain yield and in corroboration with the findings reported by Sharma et al. (2007), and Singh and Jain (2000).

## CONCLUSION

Rice varieties responded well to increasing levels of nitrogen application. Treatment of 150 Kg N ha<sup>-1</sup> has shown higher growth, maximum number of days to panicle initiation, days to 50 % anthesis and days to maturity, and recorded highest grain yield while lowest values were observed with 0 Kg N ha<sup>-1</sup>. Among the rice genotypes MTU-1010 has recorded highest grain yield with 150 Kg N ha<sup>-1</sup> and lowest grain yield was recorded in N-22 with 0 Kg N ha<sup>-1</sup>. The adequate quantity of nitrogen at the right time helped rice plants to produce maximum grain yield with better growth and development.

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## ACCOUNTING SPATIAL VARIABILITY OF SOIL pH, EC AND OC AT DIFFERENT SAMPLING INTENSITIES IN RICE CROP USING GEOSTATISTICS

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### ABSTRACT

The aim of this paper was to quantify and evaluate the spatial distribution of soil pH, EC and OC in different grid sampling intensities using descriptive statistics and geostatistics. The study was carried out in a farmer field from Machapur village of Siddipet district, Telangana. Using GPS, a 14.2 x 14.2 m grid sampling size was set, comprising 200 samples per 10 acres. In each grid positioned on the field, soil samples at a depth of 0 - 20 cm were extracted. Soil pH, EC and OC were determined. By deleting the alternative soil samples from the 200 grids (sequential sampling), sampling intensities of 100, 50, 25, and 10 were established, with a decreased number of samples per selected area (10 acres). Each soil attribute was submitted to descriptive statistical and geostatistical analysis at each sampling intensity. An experimental semivariogram was developed to understand the spatial dependence structure. The attributes pH, EC and OC showed spatial correlation in almost all sampling intensities, except for the sampling intensity of 10 for OC.

**Key words:** Geostatistics, semivariogram, sampling intensity, spatial variability

Soil is the soul of life. An intimate knowledge on their spatial location, extent, distribution characteristics, classification, is a prerequisite for agricultural practices. Soils are characterized by high degree of spatial variability due to the combined effect of physical, chemical and biological processes that operate with different intensities and at different scales. Knowledge on spatial variation of soil properties is important in several disciplines, including landuse planning, agricultural field trial research and precision farming. Both inherent and human-induced variability lead to non-uniform crop production at the field level. Thus information on spatial variability of soil properties is vital for improving soil management and hence crop productivity.

Soil variability can be defined with classic statistical methods and it is assumed as soils are having a random property (Cemek *et al.*, 2007). However, many reported that soil characteristics show spatial dependence (Costa *et al.*, 2015; Ozgoz *et al.*, 2013). In this regard, classical statistics is not capable of analyzing the spatial dependency of the

variables since the data is assumed to be measured independently (Vieira *et al.*, 1983) and it is thus possibly evaluated using a geostatistical approach (Patil, *et al.*, 2011). Earlier studies in different parts of the globe (Behera and Shukla, 2015; Xu *et al.*, 2013) proved that geostatistical analysis methods are most useful for accurate assessment and mapping the spatial variability of soil fertility of un-sampled locations in a timely and accurate manner. It has been observed that among different methods of spatial interpolation of soil properties, kriging is an optimal interpolation method.

Among statistical methods, geo statistical kriging-based techniques (Pyrzcz and Deutsch, 2014) are widely applied, this model estimate values at un-sampled locations based on the measurement at surrounding locations with certain assigned weights for each measurement. From a theoretical stand point, kriging is the optimal interpolation method; however, its correct application requires an accurate determination of the spatial structure via semivariogram construction and model-fitting. The objective of this study is to determine the degree of spatial variability of pH, EC



(Electric Conductivity) and Organic carbon (OC) with classical and geo statistical analysis for farmer field of Siddipet district of Telangana.

## MATERIAL AND METHODS

### Site Description

A farmer field from Machapur village of Siddipet district having geographic coordinates from 18°11' 06.98" to 18°11' 8.78" N latitude and 78°53' 10.67" to 78°53' 20.57" E which belongs to the Central zone of Telangana (Figure 1) which is most prominent for continuous rice growth was selected. It is having annual Rainfall of 742.7 mm and minimum-maximum average annual temperatures are 20.6°C and 33.5°C, respectively. Annual Values of relative humidity (RH %) are about 64 % to 92 % in the morning and 23 % to 65 % in the afternoon. The texture of soil is red loamy in nature.

### Soil Sampling and Analysis

A farmer field of 10 acres was selected in Machapur village of Siddipet district which is continuously under paddy cultivation. A total of 200 point soil samples were collected by making the grids of size 200 m<sup>2</sup> (14.2 m \* 14.2 m) area. Grids were prepared to get the required number of samples by using QGIS 3.8 software. In point sampling (cell center sampling) takes one sample from the center of each grid cell. The 200 single-sampled plots will be aggregated to coarser intervals (to give sample number of 100, 50, 25 and 10 in the sampled field) by nearest neighborhood selection process. The collected soil samples were analysed for pH, EC, and organic carbon (OC) in a laboratory. Soil pH was determined in 1:2.5 soil water suspensions by potentiometric method (Jackson, 1973). Electrical conductivity was determined in 1: 2.5 soil-water extract using Conductivity Bridge (Jackson, 1973). The organic carbon was determined by Walkley and Black's wet oxidation method (Walkley and Black, 1934).

## Statistical Analyses

### Classical Statistical Analysis

The main statistical parameters, including mean, median, standard deviation, variance, coefficient of variance, and maximum and minimum values, which are generally accepted as indicators of the central tendency and of the data spread, were analyzed.

These statistical parameters were calculated in Arc map 10.8.

### Geostatistical Analysis and Mapping

The structure of the spatial variability was assessed by calculating semivariograms represented in below equation (Costa *et al.*, 2015; Mohammadi, 2002).

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^n [Z(X_i) - Z(X_i + h)]^2$$

where: n is the number of pairs of sample points separated by the distance h and Z(xi)'s are the value of the characteristic under study at i<sup>th</sup> location (i = 1, 2, 3, . . . , n).

Kriging works for normally distributed data and hence the data of soil fertility parameters were log transformed to normalize the distribution wherever found necessary (Goovaerts *et al.*, 2005). Prior to mapping, well-known theoretical models such as Circular, Spherical, Gaussian, and Exponential were tested for each soil parameter data to calculate the experimental semivariogram and select the best fitted model. The models provide information about the spatial structure as well as the input parameters for interpolation. Values of root-mean-square standardized error (RMSSE), mean standard error (MSE) and root-mean-square error (RMSE) were estimated to ascertain the fitted model. The best models of the fitted models were selected on the basis of error values computed from the entire data sets (Ewis, 2012; Gorai and Kumar, 2013). Accordingly, the model is showing lowest RMSE value and RMSSE value close to one is considered as the best fitting model for interpolation (Gorai and Kumar, 2013). Next, the best models were used to analyze the spatial structure and provide the input parameters for interpolation. Then after, kriged maps showing the values of un-sampled locations were generated.

$$\begin{aligned} \text{RMSSE} &= \sqrt{\frac{1}{n} \sum_{i=1}^n \{ (Z(X_i) - \check{Z}(X_i)) / \sigma(X_i) \}^2} \\ \text{MSE} &= \sqrt{\frac{1}{n} \sum_{i=1}^n \sigma^2(X_i)} \\ \text{RMSE} &= \sqrt{\frac{1}{n} \sum_{i=1}^n (Z(X_i) - \check{Z}(X_i))^2} \end{aligned}$$

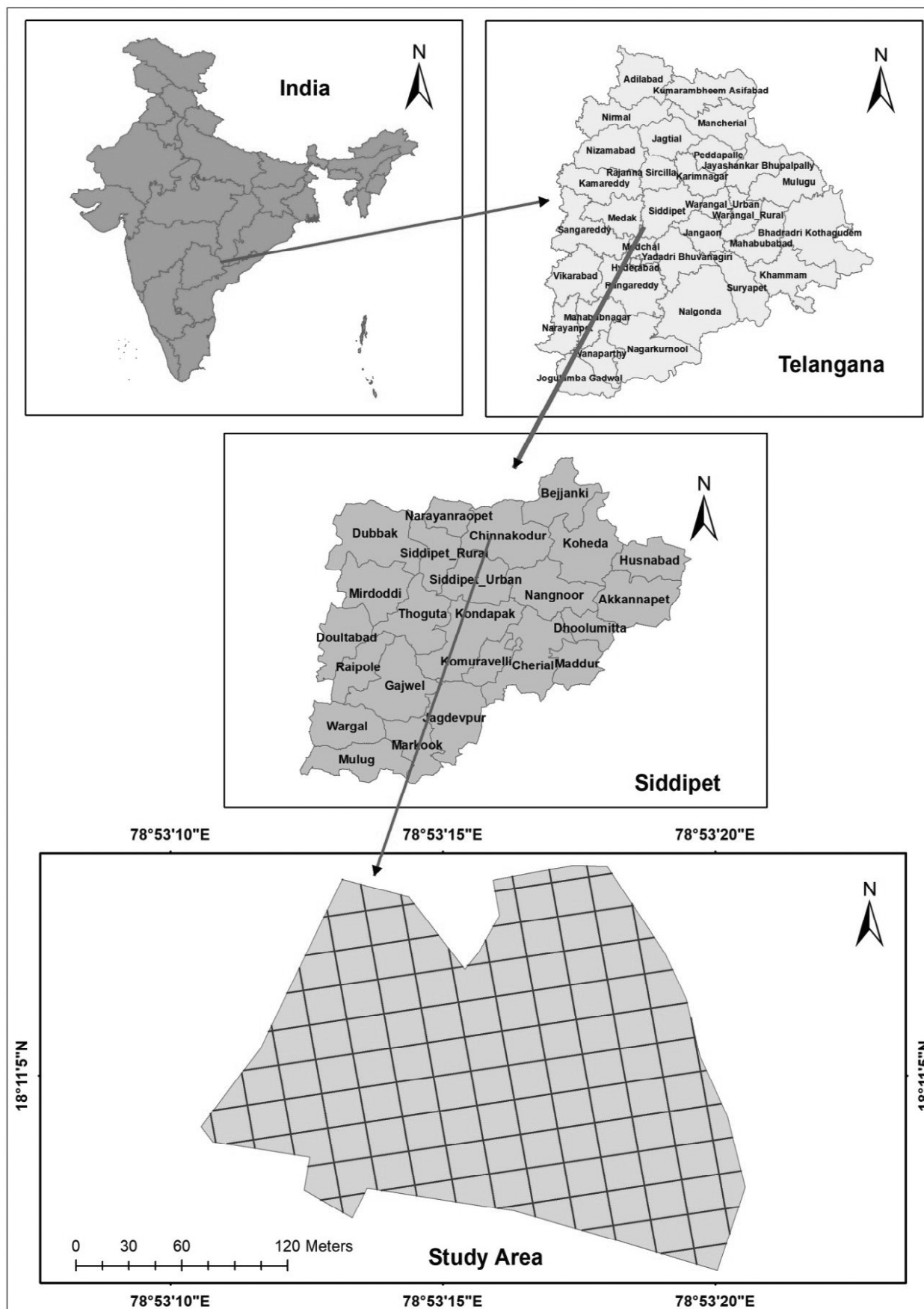


Figure 1. Location of the study area and soil sampling grid for farmer field in Machapur village of Siddipet district

Where:  $Z(X_i)$  is the value of the variable  $Z$  at the location of  $X_i$ ,  $\hat{Z}(X_i)$  is the predicted value at location  $i$ ,  $n$  is the sample size and  $\sigma^2(X_i)$  is the kriging variance for location  $X_i$ .

### Validation of Soil Maps

The performance/ effectiveness of interpolation was evaluated based on Goodness of-Prediction Estimate (G) (Karydas *et al.*, 2009; Krivoruchko and Gotay, 2003). A “G” value equal to 100 % indicates a perfect prediction, positive values (*i.e.*, 0 to 100 %) indicate that the predictions are more reliable than the use of the sample mean, and negative values indicate that the predictions are less reliable than the use of the sample mean instead.

$$G = 1 - \frac{\sum_{i=1}^n (Z(X_i) - \hat{Z}(X_i))^2}{\sum_{i=1}^n (Z(X_i) - \hat{Y})^2 * 100}$$

Where:  $Z(x_i)$  is the observed value at location  $i$ ,  $\hat{Z}(x_i)$  is the predicted value at location  $i$ ,  $n$  is the sample size and  $\hat{Y}$  is the sample mean.

## RESULTS AND DISCUSSION

### Descriptive statistics for point samples

The summary of descriptive statistics for measured soil properties of pH, EC and OC were presented in Table 1.

**Table 1. Descriptive statistics of pH, EC and OC of soil**

Sampling density	Minimum	Maximum	Mean	Median	Skewness	Kurtosis	SD	CV %
<b>pH</b>								
200	7.03	7.99	7.60	7.61	-0.21	2.85	0.18	2.37
100	7.03	7.89	7.59	7.61	-0.54	3.04	0.17	2.32
50	7.03	7.89	7.58	7.60	-0.61	3.53	0.17	2.35
25	7.26	7.85	7.60	7.62	-0.21	2.36	0.16	2.08
10	7.35	7.71	7.56	7.53	-0.29	2.09	0.12	1.60
<b>EC (dS m<sup>-1</sup>)</b>								
200	0.14	0.45	0.27	0.23	0.80	2.80	0.10	36.95
100	0.19	0.42	0.27	0.22	1.09	2.93	0.10	36.95
50	0.21	0.42	0.28	0.24	0.97	2.86	0.10	36.01
25	0.21	0.42	0.26	0.30	1.16	3.12	0.09	35.39
10	0.21	0.36	0.28	0.31	0.72	1.79	0.10	34.73
<b>OC (g kg<sup>-1</sup>)</b>								
200	3.8	6.9	5.3	5.1	0.29	2.22	1.26	23.85
100	4.6	6.5	5.2	5.0	0.46	2.32	1.17	22.43
50	4.9	6.5	5.4	5.3	0.32	2.03	1.22	22.54
25	5.3	6.5	5.6	5.4	0.57	2.33	1.26	22.43
10	5.9	6.5	5.9	5.6	0.46	1.67	1.31	22.16

### Soil pH

The mean pH values for point soil samples in the study area was recorded highest for 200 ( $7.60 \pm 0.18$ ) and 25 ( $7.60 \pm 0.16$ ) sampling intensities followed by 100 ( $7.59 \pm 0.17$ ), 50 ( $7.58 \pm 0.17$ ) and 10 ( $7.56 \pm 0.12$ ) samplings. The pH values for all sampling intensities were normally distributed as they ranged within -1 to +1 and all are negatively skewed (-0.21 to -0.61). The highest kurtosis value was observed in 50 sampling intensity (3.53), followed by 100 (3.04), 200 (2.85), 25 (2.36) and 10 (2.09), respectively. The coefficient of variation (CV) for pH was recorded maximum for 200 sampling intensity (2.37 %) followed by 2.32 %, 2.35 %, 2.08 % and 1.6 % for 100, 200, 25 and 10 sampling intensities, respectively.

### Soil EC

Irrespective of sampling intensities, the mean electrical conductivity varied from 0.26 to 0.28 dS m<sup>-1</sup> and rated as non-saline. Distribution of EC was positively skewed. Non-normal distribution was observed in 100 (1.09) and 25 (1.16) sampling intensities as exceeded skewness value of >1 whereas, normal distribution was observed in 200 (0.80), 50 (0.97) and 10 (0.72) sampling intensities. The EC was positively kurtotic and recorded the highest for sampling intensity of 25 (3.12) followed by 100

(2.93), 50 (2.86), 200 (2.80) and 10 (1.79). The EC showed more variation with high CV (>35 %) values and recorded in the order of 200 (36.95) > 100 (36.95) > 50 (36.01) > 25 (35.39) > 10 (34.73).

### Soil OC

Irrespective of the sampling size, it ranged from 3.8 to 6.9 g kg<sup>-1</sup>. The mean OC values (g/kg) with SD were recorded as 5.3 ± 1.26, 5.2 ± 1.17, 5.4 ± 1.22, 5.6 ± 1.26 and 5.9 ± 1.31 for 200, 100, 50, 25 and 10 sampling intensities. Distribution of OC was normal for all sampling sizes as skewness values ranged between 0.29 to 0.57 and kurtosis values from 1.67 to 2.33. Highest variation was observed at 200 (23.85) sampling intensity followed by 50 (22.54), 100 (22.43), 25 (22.43) and the least variation was found at 10 (22.16) sampling intensity.

Referring the mean values (Table 1), the studied soils were slightly alkaline in soil pH *i.e.*, around 7.5 and non-saline in nature. It may be due to continuous flooding in rice fields. It brings the soil pH to neutral. The soil in the studies area is red loamy texture with good drainage conditions leads the leaching of salts. The organic carbon (OC) content was rated as low to medium in sampling area. The low OC content was possibly due to the erosion of topsoil, complete crop residue removal, and high rate of organic matter decomposition due to continuous cultivation (Laekemariam *et al.*, 2016a, 2016b; Vasu *et al.*, 2017).

The coefficient of variation is the ratio of standard deviation to mean is a useful measure of overall variability. There was difference in CV of soil properties. The above table showed that, decrease in coefficient of variation with decreasing sampling intensity from 200 to 10 which indicating that homogeneity of the field. From this we can conclude that, the selected field required higher sampling intensities to know the spatial variability of selected soil parameters. The greatest variation was observed in EC (36.95 %) whereas the smallest variation of soil pH (1.60 %) at sampling density of 10. The diverse variation could be attributed to pedogenic processes influenced by the micro-topographical variations (Vasu *et al.*, 2016) and management differences (Laekemariam *et al.*, 2016a).

### Geo-Statistical Analysis for Siddipet point samples

Semivariogram analysis and characteristic parameters for soil pH, EC and OC of soil properties are presented in Table 2 and Figure 2-4.

#### Soil pH

The variogram of soil pH was best fit to circular model for both 200 (0.1573) and 50 (0.1773) sampling intensities and exponential model for 100 (0.1755) and 25 (0.1695) sampling intensities whereas gaussian model for 10 (0.0978) sampling intensity as they recorded lowest RMSE values among different models. The nugget and sill values were 0.0154 and 0.0272 at 200, 0.0087 and 0.0291 at 100, 0.0253 and 0.0351 at 50, 0.0243 and 0.0252 at 25 and 0.0077 and 0.0193 at 10 sampling intensities, respectively. The distribution of pH was weakly spatial dependent at sampling intensity of 25 with the nugget to sill ratio of 96.22 per cent and moderately spatially dependent at 200, 100, 50 and 10 sampling intensities with the nugget to sill ratio of 56.50, 29.79, 72.12 and 39.93 per cent respectively. The range estimates were 31.2, 38.8, 157.9, 316.4 and 213.4 m for 200, 100, 50, 25 and 10 sampling intensities, respectively.

#### Soil EC

Spatial distribution of electrical conductivity (EC) was best fit to circular model at 200 (0.0859) & 10 (0.0848) sampling intensities and exponential model for 100 (0.0950) & 50 (0.0972) whereas gaussian model for 25 (0.1036) sampling intensities. The distribution of EC was moderately spatial dependent at 200 sampling intensity with the nugget to sill ratio of 25.39 per cent and strongly spatially dependent at 100, 50, 25 and 10 sampling intensities with the nugget to sill ratio of 17.96, 11.79, 24.65, 0.00 per cent respectively. Ranges were 39.7, 332.4, 94.3, 243.2, and 456.6 m for 200, 100, 50, 25 and 10 sampling intensities, respectively.

#### Soil OC

The OC was best fit to gaussian model at 200 (0.1575) & 25 (0.1636) sampling intensities then exponential model for 100 (0.1588), circular model for 50 (0.1822) whereas all models were equally fitted for

Table 2. Best fitted semivariogram models for Soil pH, EC and OC

Sampling density	Transformation	Semivariogram Model	RMSE	MSE	RMSSE	G%	Nugget (C <sub>0</sub> )	partial sill (C)	Sill (C <sub>0</sub> +C)	range (m)	Spatial dependence C <sub>0</sub> (C <sub>0</sub> +C)	Spatial dependence level
<b>pH</b>												
200	NO	Circular	0.15725	0.1568	1.004	16.57	0.0154	0.0118	0.0272	31.2	56.50	Moderate
100	NO	Exponential	0.1755	0.1668	1.0494	-0.10	0.0087	0.0204	0.0291	38.8	29.79	moderate
50	NO	Circular	0.1773	0.1733	1.0212	-1.42	0.0253	0.0098	0.0351	157.9	72.12	moderate
25	NO	Exponential	0.1695	0.1635	1.0370	-20.34	0.0243	0.0010	0.0252	316.4	96.22	weak
10	NO	Guassian	0.0978	0.1152	0.8786	26.69	0.0077	0.0116	0.0193	213.4	39.93	moderate
<b>EC (dS m<sup>-1</sup>)</b>												
200	NO	Circular	0.08595	0.0856	1.008	59.00	0.0031	0.0091	0.0121	39.7	25.39	Moderate
100	LOG	Exponential	0.0950	0.0960	1.0852	52.11	0.0313	0.1430	0.1743	332.4	17.96	strong
50	NO	Exponential	0.0972	0.0949	1.0142	48.14	0.0019	0.0140	0.0158	94.3	11.79	strong
25	LOG	Guassian	0.1036	0.0995	1.0242	45.91	0.0484	0.1479	0.1963	243.2	24.65	strong
10	NO	Circular	0.0848	0.1157	0.6336	72.41	0.0000	0.0779	0.0779	456.6	0.00	strong
<b>OC (g kg<sup>-1</sup>)</b>												
200	NO	Gaussian	0.15749	0.1602	0.9839	25.65	0.0200	0.0137	0.0337	46.0	59.35	Moderate
100	NO	Exponential	0.1588	0.1641	0.9700	13.42	0.0188	0.0115	0.0303	90.1	62.02	moderate
50	NO	Circular	0.1822	0.1888	0.9680	-2.62	0.0270	0.0088	0.0358	58.3	75.47	weak
25	NO	Guassian	0.1636	0.1710	0.9792	17.69	0.0114	0.0268	0.0382	58.9	29.90	moderate
10	NO	All models equally fitting	0.2239	0.2204	1.0138	-31.32	0.0429	0.0000	0.0429	421.0	100.00	weak

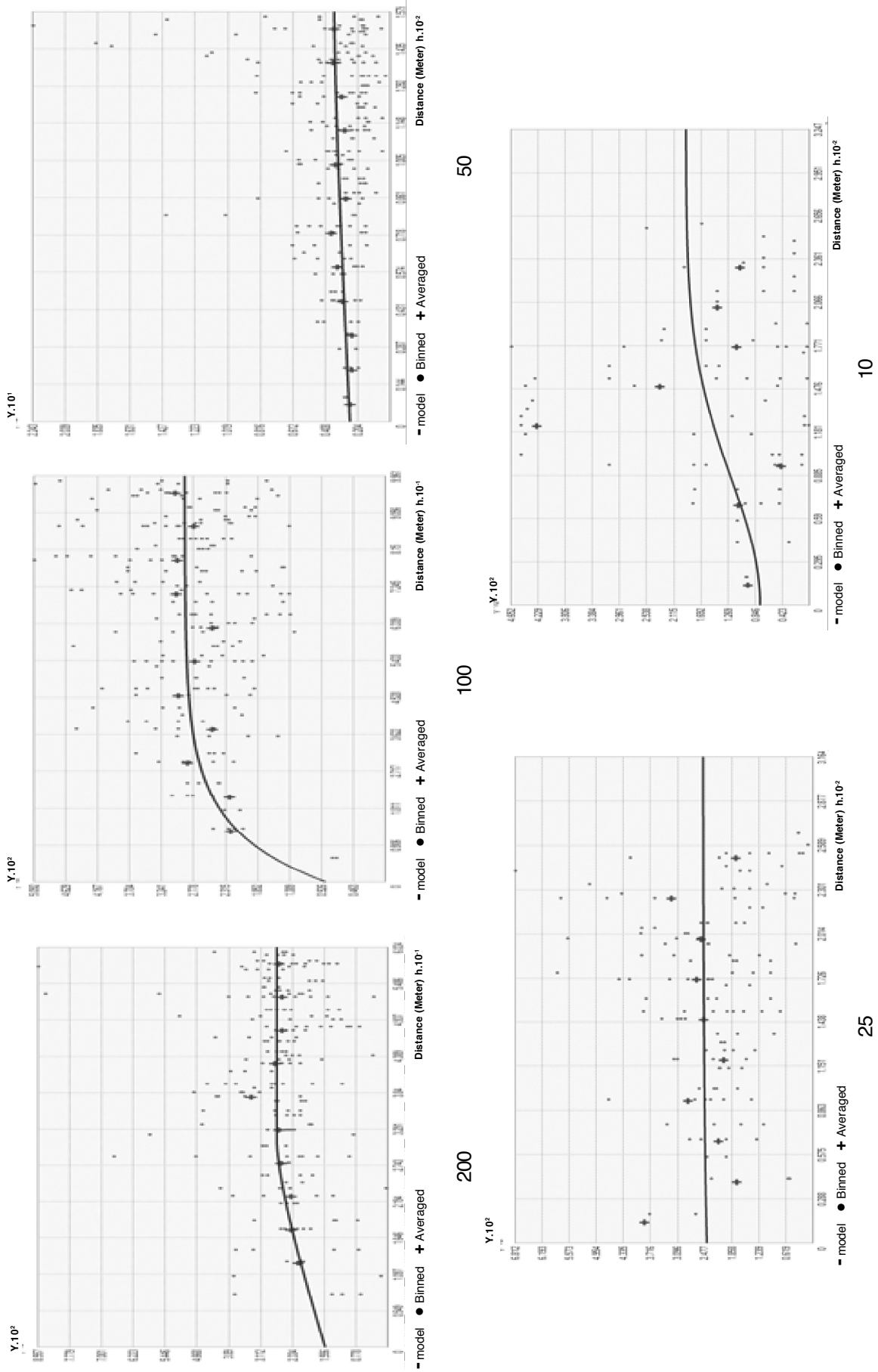


Figure 2. Semivariogram for soil pH at different sampling intensities

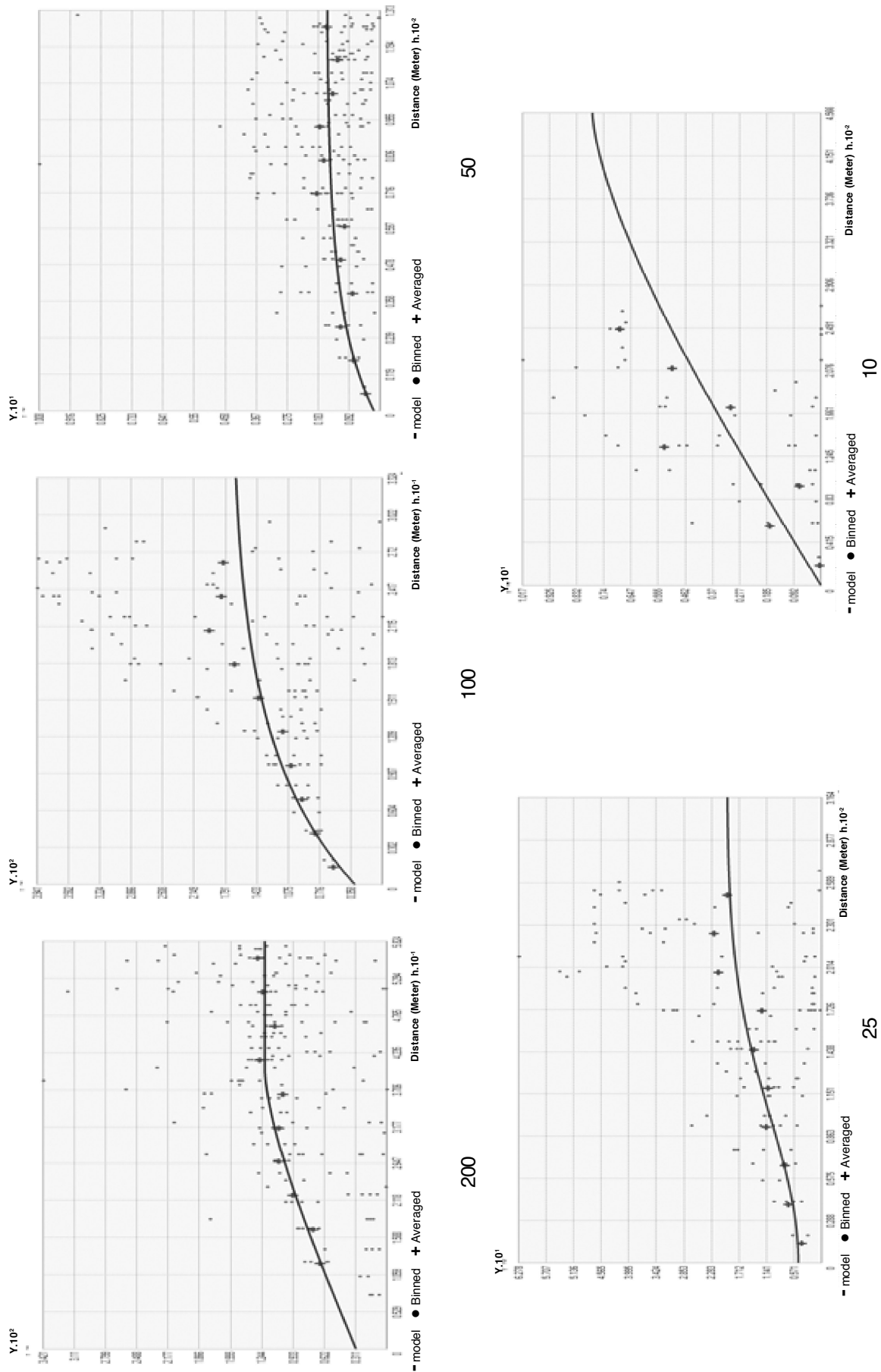
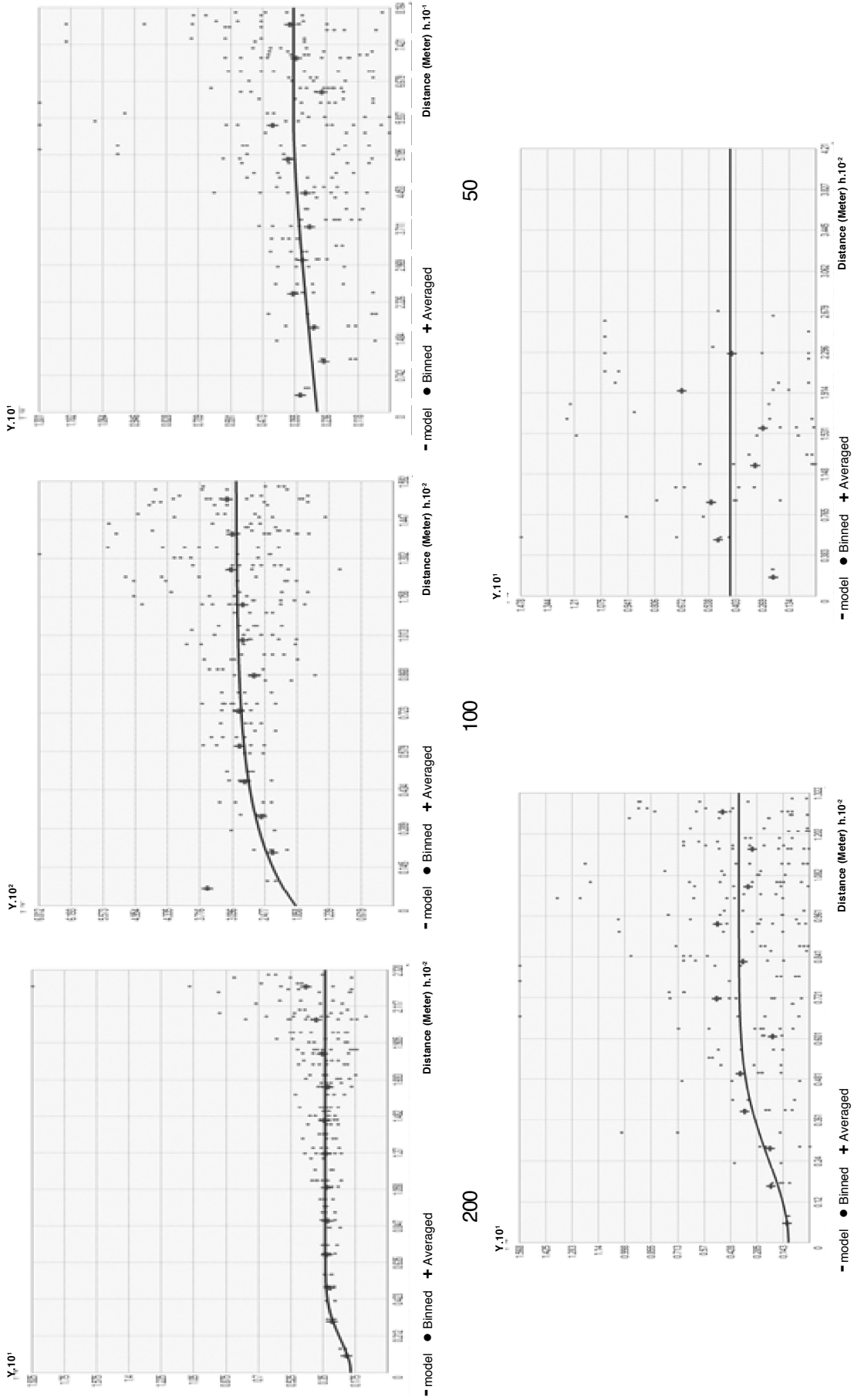


Figure 3. Semivariogram for soil EC at different sampling intensities



10

25

Figure 4. Semivariogram for soil OC at different sampling intensities



10 (0.2239) sampling intensity. The nugget variance and sills of the variogram were 0.0200 and 0.0337 at 200, 0.0188 and 0.0303 at 100, 0.0270 and 0.0358 at 50, 0.0114 and 0.0382 at 25 and 0.0429 and 0.0420 at 10 sampling intensities, respectively. Moderate spatial dependency observed at 200, 100 and 25 sampling intensities whereas weak spatial dependency was seen at 50 and 10 sampling intensities. Ranges were, 46.0, 90.1, 58.3, 58.9 and 421.0 m for 200, 100, 50, 25 and 10 sampling intensities, respectively.

#### Cross validation of spatial variability maps for point samples in Siddipet

The goodness of prediction (G) coefficient of soil pH was varied from -31.32 to 26.69 per cent, whereas it was ranged from 45.91 to 32.41, -31.32 to 25.65 for soil EC and OC respectively (Table 3). The average percent G values for all analyzed attributes

indicating that the interpolation model used for nutrient mapping was suitable. The conclusion was that taking the more number of samples may lead the better estimation of spatial variability.

The ratio between nugget and sill expressed as a percentage was used to determine the strength of the spatial dependence of soil properties. The spatial dependency varied from nil to 100% (Table 2). Based on the work of Cambardella *et al.*, (1994), the ratio < 25%, 25–75% and > 75% suggests a strong, moderate and weak spatial dependency, respectively. A strong spatial dependence is associated to the variation of intrinsic soil properties such as soil parent material, topography, texture, and mineralogy (Xu *et al.*, 2013; Behera and Shukla, 2015; Costa *et al.*, 2015). On the other hand, a weak spatial dependence of soil properties indicated that the spatial variability was

**Table 3. Goodness of prediction (G%) for spatial variability maps of soil pH, EC and OC**

Variables Sampling densities	G %				
	200	100	50	25	10
pH	16.57	0.10	-1.42	-20.34	26.69
EC (dS m <sup>-1</sup> )	59.00	52.11	48.14	45.91	32.41
OC (g kg <sup>-1</sup> )	25.65	13.42	-2.62	17.69	-31.32
Average G%	33.74	21.88	14.70	14.42	9.26

(pH, EC and OC) were 33.74, 21.88, 14.70, 14.42 and 9.26 at 200, 100, 50, 25 and 10 sampling intensities.

The goodness of prediction (G) coefficient of the study for all estimated soil properties was positive at higher sampling intensities such as 200 and 100. In most of the cases it got decreased with the decreasing sampling intensity in all of three soil parameters. Similar results were observed by Kerry and Oliver (2008) and Nanni *et al.*, (2011). The positive coefficients signified that interpolation technique and predictions are more reliable than using the sample means (Karydas *et al.*, 2009). The sampling intensities which were shown negative G % in the exploratory analysis did not reveal any kind of tendency and it was not easy to detect any spatial arrangement. Therefore, it should have been excluded in advance from further analysis. The rest of the soil properties had positive G values,

mainly attributed by extrinsic variations such as soil fertilization and cultivation practices (Cambardella *et al.*, 1994; Guan *et al.*, 2017; Ozgoz *et al.*, 2013). A moderate spatial dependence is likely to be controlled by both intrinsic and extrinsic factors (Behera and Shukla, 2015; Costa *et al.*, 2015; Guan *et al.*, 2017).

In this study, all the three properties (pH, EC and OC) have shown the different spatial distribution at different sampling intensities (Table 2). The moderate spatial dependency of soil OC may be attributed to poor management practices such as lack of addition of crop residues and organic manures. And also intensive cropping and high temperatures increases the mineralization of OC. The strong spatial dependency of EC may be due to non-saline parent material. Continuous flooded condition as well as neutral parent materials may be the reasons for moderate spatial dependency of soil pH.

## CONCLUSION

Descriptive statistics of the study area attributes analyzed together with different sampling intensities showed that chemical homogeneity with low sampling intensities as they were recorded the low percent CV values than compared to higher sampling intensities. The results obtained for each attribute in their regular sampling intensities concluded that higher sampling intensities can satisfactorily represent the spatial variability of the study area. In the semivariogram analyzes, all the three attributes presented showed the spatial dependence with exceptions: OC at sampling intensity of 10 which showed the pure nugget effect. It is indicating that the sampling intensity to determine the spatial distribution pattern is dependent on the soil attribute studied.

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## ACCOUNTING SPATIAL VARIABILITY OF SOIL PH, EC AND OC

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## RESPONSE OF IN-SITU PADDY STRAW MANAGEMENT PRACTICES ON MICROBIAL ACITVITY, ENZYMATIC ACTIVITY AND CROP PRODUCTIVITY IN SUBSEQUENT ZERO TILL MAIZE

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### ABSTRACT

Rice (*Oryza sativa*) - Maize (*Zea mays* L.) is one of the predominant cropping system of Telangana. The concept of zero tillage is gaining momentum in traditional areas under rice-maize sequence. The intensification of rice based cropping systems is resulting in a larger volume of rice straw being produced that, in turn, must be managed over shorter turnaround times between crops. The present study was conducted at College farm, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University during *rabi* 2020-21 and 2021-22 with eight residue management practices (M<sub>1</sub>: Burning residue before sowing, M<sub>2</sub>: Retention of residue, M<sub>3</sub>: Removal of residue before sowing, M<sub>4</sub>: Incorporation at 15 DAS, M<sub>5</sub>: Incorporation at 15 DAS + SSP at equivalent to 'P' dose, M<sub>6</sub>: Spraying consortia of decomposers @ 10% of residue weight + surface retention, M<sub>7</sub>: Spraying consortia of decomposers @ 10% of residue weight + incorporation at 15 DAS, M<sub>8</sub>: Spraying consortia of decomposers @ 10% of residue weight + incorporation at 15 DAS + SSP at equivalent to 'P' dose and three fertility levels (S<sub>1</sub>: 75 % RDF, S<sub>2</sub>: 100% RDF and S<sub>3</sub>: 125 % RDF) laid out in strip plot design with three replications in both the years. The results of the study revealed that higher grain yield (6601 and 6999) and straw yield (8151 and 8555) were obtained with incorporation + consortium + SSP treatment in combination with 75 % RDF during *rabi* 2020-21 and 2021-22, respectively. Similarly bacterial and fungi population with higher dehydrogenase activity were recorded under incorporation + consortium + SSP treatment along with 125 % RDF in *rabi* 2020-21 and 2021-22. Hence, it can be concluded that incorporation of crop residues with SSP application equivalent to 'P' dose and microbial consortium along with the addition of 75 % RDF performed better than mere incorporation, removal, retention, retention + consortia and burning of residues in combination with 125 % RDF.

**Keywords:** Consortia, microbial count, residue management, yield, zero till maize

Maize is called as 'miracle' crop and also 'queen of cereals' because of its high yield potential and wider adaptability. In India, maize is considered as third most important crop among the cereals and used as a staple food in many developing countries. Worldwide, maize is grown in an area of 197.20 Mha with a production of 1148.49 Mt and productivity of 5824 kg ha<sup>-1</sup> (FAOSTAT, 2019-20) while 9.56 M ha with 28.77 Mt production and 3006 kg ha<sup>-1</sup> productivity in our country. In Telangana, maize occupies an area of 0.56 M ha with production and productivity of 2.99 Mt and 534 kg ha<sup>-1</sup> respectively (CMIE, 2019-20). Maize yields in India need to be increased significantly so as to meet food, feed and industrial needs.

Adoption of resource conserving tillage practices in India is mostly taking place in rice-wheat system and is quite less in other prominent cropping

systems. Rice-maize rotation is one of the major cropping systems in Telangana. For the rapid expansion of rice-maize system, sustainable and cost effective technologies with reduced labour requirement and rapid turn around between crops are needed. Potential technologies include zero tillage and crop residue addition. Crop residues are plant parts that are left in the field after harvesting of a crop. Large quantities of residues are generated every year in agriculture. They are the potential natural resources that alters the soil environment, which in turn influences the soil microbial activity and subsequent nutrient transformations. Crop residue burning and imbalanced use of chemical fertilizers in intensive crop rotations are the important ecological threats in any agro-ecosystem. From the farmers' point of view, burning may be seen as a method of disposing crop residues. While open-field

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burning may have positive effects on managing pests, it leads to loss of nutrients and creates air pollution that causes human respiratory ailments. So there is a dire need to adopt ways and means to manage this valuable resource.

More sustainable rice straw management methods are urgently needed to minimize rice production's carbon footprint and its negative effects on human health and to add value to the straw byproduct. With this background, present investigation has been proposed to know the influence of residue management practices and fertility levels on microbial status and productivity of zero till maize.

## MATERIAL AND METHODS

A field experiment was conducted during *rabi* 2020-21 and 2021-22 at Agricultural College Farm, Professor Jayashankar Telangana State Agricultural University with twenty four treatments laid out in strip plot design with three replications. The soil of the experimental site was sandy clay loam in texture, neutral in reaction (pH 7.88), low in organic carbon (0.20 %) and available nitrogen (172 kg ha<sup>-1</sup>), medium in available phosphorous (22.21 kg ha<sup>-1</sup>) and high in available potassium (398.16 kg ha<sup>-1</sup>). Treatments included residue management options M<sub>1</sub>: Burning residue before sowing, M<sub>2</sub>: Retention of residues, M<sub>3</sub>: Removal of residues before sowing, M<sub>4</sub>: Incorporation at 15 DAS, M<sub>5</sub>: Incorporation at 15 DAS + SSP at equivalent to 'P' dose, M<sub>6</sub>: Spraying consortia of decomposers @ 10% of residue weight + surface retention, M<sub>7</sub>: Spraying consortia of decomposers @ 10% of residue weight + incorporation at 15 DAS, M<sub>8</sub>: Spraying consortia of decomposers @ 10% of residue weight + incorporation at 15 DAS + SSP at equivalent to 'P' dose and three fertility levels (S<sub>1</sub>: 75 % RDF, S<sub>2</sub>: 100% RDF and S<sub>3</sub>: 125 % RDF). Maize variety 'DHM-117' was used for the experiment after the harvest of rice under zero till conditions. Recommended dose of fertilizer was 240:80:80 kg N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O as urea, single super phosphate and murate of potash was applied as per the treatments. The entire P<sub>2</sub>O<sub>5</sub> and half of K<sub>2</sub>O were applied at sowing. Nitrogen was applied in three equal splits (1/3rd each at basal, knee-high and tasseling). Similarly, the remaining potassium was applied along with urea during second top dressing at tasseling. Gross plot size and net plot size were 5.4

m x 4.0 m and 3.0 m x 3.2 m respectively during both seasons. The data pertaining to microbial count and enzyme activity was obtained from the collection of soil samples from plant rhizosphere at 50 % flowering and harvest and analyzing the fresh samples within 24 hours. Colony forming units of bacteria (X 10<sup>6</sup> CFU g<sup>-1</sup>soil) and fungi (X 10<sup>4</sup> CFU g<sup>-1</sup>soil) were determined by serial dilution and plating onselectivemedia. The replicates of the inoculated agar plates were incubated for two days at 37 °C for bacteria and five days for fungi at 28 °C. Number of colonies on plates were recorded and population per gram soil was enumerated by using digital colony counter. The number of colonies were multiplied by the dilution factor and expressed as number of colony forming units (CFU) per gram of soil. For analysis of dehydrogenase activity, one gram of soil sample was taken in 50 ml glass tube and 50 mg of CaCO<sub>3</sub> was added followed by 2.5 ml of distilled water and 1ml of 3% 2,3,5-triphenyl tetrazolium chloride. Swirled for few minutes and incubated at 37°C for 24 hours. The red precipitate of the TPF was dissolved in 10 ml of methanol and the contents were shaken for 30 minutes, filtered and the volume is made up to 25 ml with methanol. Intensity of red colour was measured with double beam UV-Visible spectrophotometer at 485 nm (Casida *et al.*, 1964).

For the determination of grain yield, the kernels from the air-dried cobs from each net plot were separated, cleaned and dried to obtain at least 14 per cent moisture. Weight of grains of each plot was recorded separately and expressed as grain yield in kg ha<sup>-1</sup>. Stover from the net plot area was weighed after proper sun drying and indicated as stover yield in kg ha<sup>-1</sup>.

## RESULTS AND DISCUSSION

**Microbial Population:** Microbial count was recorded from samples collected from the rhizosphere (0-15 cms) and presented hereunder.

### Bacteria

Bacterial population enumeration of soil is an indicator of soil health. From Tables 1, 2, 3 and 4, it can be concluded that both residue management and fertility levels had significant effect on bacterial population during both the years at 50 % flowering and harvest. Among the residue management

treatments, the bacterial population was significantly higher in the treatment where the residues were added than in the residue removal and burning plots. Application of consortium + SSP + incorporation of residues ( $M_8$ ) increased the microbial counts. Lower bacterial population count was recorded in *in-situ* burning of residues before sowing. Heat produced during burning probably affected microflora of the top soil as shown by Yadav *et al.*, (2009).

Among the fertility levels, the maximum colony forming units were observed with 125 % RDF which was followed by 100 % RDF and 75 % RDF. However, the lower population of bacteria was recorded as compared to fungi.

The interaction effect on bacterial count was significantly influenced by both residue management practices and fertility levels. At 50 % flowering, significantly higher bacterial population was recorded with the combination of  $M_8S_3$  (incorporation + consortia + SSP with 125 % RDF) which was on par with incorporation + consortia + SSP with 100 % RDF and incorporation + consortia + SSP with 75 % RDF. Similar trend was observed with combination of  $M_7S_3$  (incorporation + consortia with 125 % RDF) and  $M_5S_3$  (incorporation + SSP with 125 % RDF). The other treatments which follow in descending order are  $M_4S_1 > M_6S_1 > M_2S_1 > M_3S_1 > M_1S_1$ . Significantly lowest bacterial population was recorded with the treatment combination of  $M_1S_1$  (*in-situ* burning with 75 % RDF). At harvest, similar interaction effect was observed.

### Fungi

Apart from bacteria, fungal population was also assessed at 50 % flowering and harvest and presented in Tables 1, 2, 3 and 4.

A perusal of the data indicates that the fungal population ( $\times 10^4$  CFU  $g^{-1}$  soil) was also influenced by residue management and fertility levels. Similar to bacteria, the population of fungi was also higher with incorporation + consortia + SSP ( $M_8$ ) at 50 % flowering and harvest. It was superior to all other treatments. The second best treatment was incorporation + consortia ( $M_7$ ) which was again superior to incorporation + SSP ( $M_5$ ). *In-situ* burning ( $M_1$ ) was the least with respect to fungal population significantly inferior to removal ( $M_3$ ) as well.

The superiority of incorporation + consortia + SSP ( $M_8$ ) in microbial population (bacteria and fungi) might be due to the fact that addition of SSP and microbial consortia might have served as source of carbon and energy for microorganisms. *In-situ* burning ( $M_1$ ) recorded significantly less number of microbial counts. This suggests that addition of consortia and SSP is must for maintaining microbial population. In retention + consortia, even though the population was higher than retention, removal and burning but was inferior to incorporation + consortia + SSP ( $M_8$ ). This can be attributed to the fact that even though microbes are added in the form of consortia the quantity might not be sufficient to enhance the microbial population with out incorporation and SSP. Higher dose of fertilizers resulted in higher fungal population with 125 % RDF as compared to 100 % RDF and 75 % RDF.

Regarding the interaction effect during 2020-21 and 2021-22, significantly higher fungal population was found in plots applied with 125 % RDF + incorporation + SSP + consortia which remained at par with other treatments *viz.*, incorporation + consortia + SSP with 100 % RDF and incorporation + consortia + SSP with 75 % RDF at 50 % flowering and harvest stage respectively. Shukla *et al.*, (2010) also quoted that integrating microbial consortia with NPK fertilizers proved effective in improving soil microbial population.

### Dehydrogenase activity

Among all enzymes in the soil environment, dehydrogenase are the most important and extensively used indicators of overall soil microbial activity, because they occur intracellular in all living microbial cells. Dehydrogenase play a significant role in the biological oxidation of soil organic matter.

The activity of dehydrogenase was also assayed at 50 % flowering and harvest and the data is presented in Table 1, 2, 3 and 4.

In general, peak activity of this enzyme was observed at 50 % flowering which reduced at harvest.

Among the residue management methods, higher activity of dehydrogenase enzyme was observed in incorporation + consortia + SSP at flowering and harvest, which was superior to the all

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Table 1. Microbial population at 50 % flowering of maize as influenced by residue management and fertilizer levels during 2020-21.

Treatment	Bacteria (10 <sup>6</sup> CFU/g soil)				Fungi (10 <sup>4</sup> CFU/g soil)				Dehydrogenase ( $\mu$ g TPF/g/day)			
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
<b>Residue management</b>												
<b>Burning (M1)</b>	12	14	16	14	20	23	26	23	20.0	22.4	24.5	22.3
<b>Retention (M2)</b>	15	16	18	16	25	27	30	27	24.0	25.2	27.4	25.5
<b>Removal (M3)</b>	13	15	17	15	23	25	27	25	21.8	24.4	26.0	24.1
<b>Incorporation (M4)</b>	16	18	20	18	29	31	34	31	26.8	28.4	30.3	28.5
<b>Incorporation + SSP (M5)</b>	18	23	24	22	32	34	35	34	29.0	33.2	33.5	31.9
<b>Retention + consortium (M6)</b>	15	17	19	17	27	29	31	29	24.5	26.4	29.1	26.7
<b>Incorporation + SSP (M7)</b>	24	24	25	24	37	38	38	38	33.3	33.8	34.1	33.7
<b>Incorporation + consortium + SSP (M8)</b>	25	26	26	26	39	40	40	40	35.5	35.7	36.4	35.9
<b>Mean</b>	17	19	21		29	31	33		26.9	28.7	30.2	
<b>For comparison the mean of</b>	SEM $\pm$			CD	SEM $\pm$			CD	SEM $\pm$			CD
<b>Residue management</b>	0.32			0.98	0.40			1.22	0.30			0.93
<b>Fertilizer levels</b>	0.28			1.11	0.40			1.58	0.23			0.93
<b>Main plot at same level of sub plot</b>	0.34			1.00	0.34			1.00	0.3			0.87
<b>Sub plot at same level of main plot</b>	0.98			2.85	0.99			2.88	0.82			2.38

Table 2. Microbial population at harvest of maize as influenced by residue management and fertilizer levels during 2020-21.

Treatment	Bacteria (10 <sup>6</sup> CFU/g soil)				Fungi (10 <sup>4</sup> CFU/g soil)				Dehydrogenase (µg TPF g/day)			
	Fertilizer levels											
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
<b>Burning (M1)</b>	7	10	14	10	16	20	23	20	18.9	21.9	23.1	21.3
<b>Retention (M2)</b>	10	12	15	12	22	24	26	24	21.8	26.2	28.1	25.4
<b>Removal (M3)</b>	9	11	13	11	20	23	25	23	21.6	23.0	24.8	23.1
<b>Incorporation (M4)</b>	14	16	18	16	26	30	32	29	25.9	29.1	31.3	28.8
<b>Incorporation + SSP (M5)</b>	16	18	19	18	30	32	32	31	26.7	31.1	31.9	29.9
<b>Retention + consortium (M6)</b>	11	14	16	14	24	27	29	27	24.3	26.2	27.8	26.1
<b>Incorporation + SSP (M7)</b>	19	20	20	20	33	33	34	33	31.4	31.8	32.3	31.8
<b>Incorporation + consortium + SSP (M8)</b>	22	23	23	23	35	36	36	36	32.8	33.2	33.7	33.2
<b>Mean</b>	14	16	17		26	28	30		25.4	27.8	29.1	
<b>For comparison the mean of</b>	SEM±	SEM±	SEM±	CD	SEM±	SEM±	SEM±	CD	SEM±	SEM±	SEM±	CD
<b>Residue management</b>	0.13	0.13	0.13	0.42	0.30	0.30	0.30	0.92	0.15	0.15	0.15	0.46
<b>Fertilizer levels</b>	0.25	0.25	0.25	0.98	0.31	0.31	0.31	0.44	0.21	0.21	0.21	0.83
<b>Main plot at same level of sub plot</b>	0.30	0.30	0.30	0.89	0.40	0.40	0.40	1.17	0.34	0.34	0.34	0.98
<b>Sub plot at same level of main plot</b>	0.99	0.99	0.99	2.88	1.21	1.21	1.21	3.51	1.06	1.06	1.06	3.07



Table 3. Microbial population at 50 % flowering of maize as influenced by residue management and fertilizer levels during 2021-22.

Treatment	Bacteria (10 <sup>6</sup> CFU/g soil)				Fungi (10 <sup>4</sup> CFU/g soil)				Dehydrogenase (µg TPF/g/day)			
					Fertilizer levels							
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
Burning (M <sub>1</sub> )	15	18	20	18	19	24	28	24	24	26	28	26
Retention (M <sub>2</sub> )	20	22	25	22	29	30	35	31	27	31	35	31
Removal (M <sub>3</sub> )	18	20	23	20	24	27	29	27	24	29	32	28
Incorporation (M <sub>4</sub> )	23	25	27	25	34	36	38	36	32	36	38	35
Incorporation + SSP (M <sub>5</sub> )	24	27	29	27	37	40	40	39	34	39	39	37
Retention + consortium (M <sub>6</sub> )	18	22	27	22	31	34	36	34	30	33	36	33
Incorporation + SSP (M <sub>7</sub> )	28	29	29	29	41	42	42	42	38	38	39	38
Incorporation + consortium + SSP (M <sub>8</sub> )	30	31	31	31	43	44	44	44	39	40	40	40
Mean	22	24	26		32	35	37		31	34	36	
For comparison the mean of Residue management	SEM±	SEM±	SEM±	CD	SEM±	SEM±	SEM±	CD	SEM±	SEM±	SEM±	CD
Fertilizer levels	0.53	0.30	1.62	1.62	0.23	0.23	0.70	0.70	0.23	0.23	0.71	0.71
Main plot at same level of sub plot	0.30	0.49	1.19	1.19	0.32	0.39	1.28	1.28	0.51	0.47	1.38	1.38
Sub plot at same level of main plot	1.21	3.53	1.23	3.56	1.62	1.42	1.13	3.56	1.62	1.62	4.71	4.71

Table 4. Microbial population at harvest of maize as influenced by residue management and fertilizer levels during 2021-22.

Treatment	Bacteria (10 <sup>6</sup> CFU/g soil)				Fungi (10 <sup>4</sup> CFU/g soil)				Dehydrogenase (µg TPF/g/day)			
					Fertilizer levels							
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
<b>Burning (M<sub>1</sub>)</b>	11	13	15	13	19	25	29	24	20.8	24.4	26.2	23.8
<b>Retention (M<sub>2</sub>)</b>	13	17	21	17	25	28	30	28	23.8	27.7	29.3	26.9
<b>Removal (M<sub>3</sub>)</b>	12	14	17	14	24	27	29	27	22.4	24.8	27.3	24.8
<b>Incorporation (M<sub>4</sub>)</b>	20	22	24	22	31	33	35	33	28.1	31.4	34.3	31.3
<b>Incorporation + SSP (M<sub>5</sub>)</b>	22	24	25	24	33	35	35	34	31.8	34.5	34.8	33.7
<b>Retention + consortium (M<sub>6</sub>)</b>	18	20	22	20	28	31	33	31	26.1	29.2	31.1	28.8
<b>Incorporation + SSP (M<sub>7</sub>)</b>	27	26	27	27	36	37	37	37	35.5	36.1	36.7	36.1
<b>Incorporation + consortium + SSP (M<sub>8</sub>)</b>	28	29	29	29	39	39	40	39	37.3	38.1	38.3	37.9
<b>Mean</b>	19	21	23		29	32	34		28.2	30.8	32.3	
<b>For comparison the mean of</b>	SEM±			CD	SEM±			CD	SEM±			CD
<b>Residue management</b>	0.17			0.52	0.22			0.69	0.50			0.71
<b>Fertilizer levels</b>	0.39			1.54	0.23			0.92	0.28			1.12
<b>Main plot at same level of sub plot</b>	0.41			1.21	0.48			1.40	0.44			1.30
<b>Sub plot at same level of main plot</b>	1.39			4.04	1.46			4.24	1.09			3.18

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other methods. Lower dehydrogenase activity was recorded with *in-situ* burning of residue ( $M_1$ ). Several workers reported the improved dehydrogenase activity with the application of residues (Mangalassery *et al.*, 2015; Bera *et al.*, 2018 and Saikia *et al.*, 2019).

With respect to fertilizer levels, 125 % RDF registered higher dehydrogenase activity as compared to 100 % RDF and 75 % RDF.

The interaction effect when tested with residue management and fertility levels was distinctly noticeable with the treatment combinations tested. Among the treatment combinations,  $M_8S_3$  (incorporation + consortia+ SSP with 125 % RDF) recorded significantly higher dehydrogenase activity and which was statistically at par with that of  $M_8S_2$  and  $M_8S_1$ , i.e., incorporation + consortia+ SSP with 100 % RDF and incorporation + consortia+ SSP with 75 % RDF. On the other hand, the significantly lowest dehydrogenase activity was recorded with the treatment combination of  $M_1S_1$  (*in-situ* burning with 125 % RDF) but was statistically superior with that of  $M_1S_2$  (*in-situ* burning with 100 % RDF) and  $M_1S_1$  (*in-situ* burning with 75 % RDF) combinations.

The higher dehydrogenase activity under (incorporation + consortia+ SSP with 125 % RDF) was attributed to efficient use of applied nutrients under favourable growing condition as expressed by higher grain yields. Whereas, the recommended dose of nutrients and 75 % RDF had also shown the higher dehydrogenase activity due to higher microbial population and improved soil health in the present study.

### Grain yield (kg ha<sup>-1</sup>)

Data pertaining to grain yield (6601 and 6999 kg ha<sup>-1</sup>) during 2020-21 and 2021-22, respectively is furnished in Table 5 and 6. It is evident from the data that it is influenced by the residue management methods, fertility levels and their interaction.

Significantly higher grain yield was recorded in incorporation + consortia + SSP compared to all the residue management methods. On average, there was an increase of 23 and 26 % in incorporation + consortia + SSP over *in-situ* burning, during 2020-21 and 2021-22, respectively. It is clearly evident that the yield of maize was inferior in retention, removal, *in-situ*

burning and retention + consortia compared to incorporation. Higher yields in incorporation treatments might be due to better crop growth and microbial activity owing to better decomposition of residues and higher availability of nutrients which was evident in the present study. In turn, the superiority of incorporation + consortia + SSP over bare incorporation is attributed to provision of carbonic substrate through organic matter which is congenial for microbial growth (Table 4) and resulted in nutrient transformations is revealed from improved enzyme activity in the soil (Table 4).

These results are supported by the findings of several research workers (Arcand *et al.*, 2016; Choudhary *et al.*, 2016 and Govaerts *et al.*, 2007). Particularly, these experiments revealed the beneficial effects of integrated use of residues and consortia.

Even though the retention + consortia registered higher microbial populations and their activity demonstrated in the present study, the magnitude of improvement might not be sufficient to result in increased growth and yield of the crop. However, some scientists inferred that the addition of microbial consortia and SSP should be practiced for long term for the realization of economic and ecological benefits (Liu *et al.*, 2015 and Singh *et al.*, 2004).

With regard to fertility levels, significantly higher grain yield was obtained with 125 % RDF followed by 100 % RDF and 75 % RDF.

The interaction effect when tested with residue management practices and fertility levels was markedly distinctive with the treatment combinations tested. Among all the tested combinations,  $M_8S_3$  (incorporation + consortia + SSP with 125 % RDF) recorded significantly higher straw yield but it was statistically at par with that of  $M_8S_2$  (incorporation + consortia + SSP with 100 % RDF) and  $M_8S_1$  (incorporation + consortia + SSP with 75 % RDF) treatment combinations. On the other hand, the significantly lower straw yield was recorded with the treatment combination of  $M_3S_3$  (removal with 125 % RDF) which was on par with that of  $M_1S_3$  (*in-situ* burning with 125 % RDF),  $M_2S_3$  (retention with 125 % RDF) and  $M_6S_3$  (retention + consortia with 125 % RDF) combinations.

Table 5. Grain and straw yield (Kg ha<sup>-1</sup>) of maize as influenced by residue management and fertilizer levels during 2020-21.

Treatment	Grain yield (Kg ha <sup>-1</sup> )				Straw yield (Kg ha <sup>-1</sup> )			
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
<b>Residue management</b>								
Burning(M <sub>1</sub> )	4412	5161	5991	5188	5030	6232	7052	6105
Retention (M <sub>2</sub> )	4488	5438	5869	5265	4295	6245	7941	6160
Removal(M <sub>3</sub> )	4039	5255	6220	5171	5284	6180	6666	6043
Incorporation (M <sub>4</sub> )	4564	5418	6239	5407	4649	6553	8061	6421
Incorporation + SSP (M <sub>5</sub> )	5381	5923	6088	5797	5611	7492	7956	7020
Retention + consortium (M <sub>6</sub> )	4724	5355	5941	5340	5537	6254	7268	6353
Incorporation + SSP (M <sub>7</sub> )	6132	6211	6273	6205	7508	7581	7702	7597
Incorporation + consortium + SSP (M <sub>8</sub> )	6487	6529	6787	6601	7932	8164	8357	8151
Mean	5028	5661	6176		5731	6838	7625	
For comparison the mean of	SEM±	SEM±	SEM±	CD	SEM±	SEM±	SEM±	CD
Residue management	125.2	125.2	379.8		159.1	159.1	482.7	
Fertilizer levels	127.7	127.7	501.6		184.0	184.0	722.5	
Main plot at same level of sub plot	139.9	139.9	405.3		217.9	217.9	631.2	
Sub plot at same level of main plot	413.9	413.9	1199.1		666.9	666.9	1932.1	

Table 6. Grain and straw yield (Kg ha<sup>-1</sup>) of maize as influenced by residue management and fertilizer levels during 2021-22.

Treatment	Grain yield (Kg ha <sup>-1</sup> )				Straw yield (Kg ha <sup>-1</sup> )			
	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean	75 % RDF (S <sub>1</sub> )	100 % RDF (S <sub>2</sub> )	125 % RDF (S <sub>3</sub> )	Mean
<b>Residue management</b>								
Burning(M <sub>1</sub> )	4828	5210	5807	5282	5170	6396	7219	6262
Retention (M <sub>2</sub> )	4909	5359	5723	5330	4816	6417	7797	6343
Removal(M <sub>3</sub> )	4520	5403	5801	5241	4352	6221	8041	6205
Incorporation (M <sub>4</sub> )	5383	5771	6292	5815	6206	6873	7887	6989
Incorporation + SSP (M <sub>5</sub> )	5867	6291	6489	6216	6970	7750	7814	7511
Retention + consortium (M <sub>6</sub> )	4976	5377	5854	5402	5060	6490	7839	6463
Incorporation + SSP (M <sub>7</sub> )	6518	6631	6693	6614	7915	8055	8143	8038
Incorporation + consortium + SSP (M <sub>8</sub> )	6929	6971	7096	6999	8269	8568	8828	8555
Mean	5491	5877	6219		6095	7096	7946	
For comparison the mean of	SEM±	SEM±	CD	CD	SEM±	SEM±	CD	CD
Residue management	125.1	125.1	379.7	379.7	169.2	169.2	513.3	513.3
Fertilizer levels	82.8	82.8	325.2	325.2	167.4	167.4	657.3	657.3
Main plot at same level of sub plot	100.1	100.1	290.0	290.0	218.2	218.2	632.3	632.3
Sub plot at same level of main plot	238.7	238.7	691.7	691.7	647.4	647.4	1875.4	1875.4

### Straw yield (kg ha<sup>-1</sup>)

The data on straw yield (8151 and 8555 kg ha<sup>-1</sup>) during *rabi*, 2020-21 and 2021-22, respectively, as influenced by residue management and fertility levels are presented in Table 5 and 6.

The straw yield (kg ha<sup>-1</sup>) of maize attained after harvest was significantly influenced by different residue management. Among the different residue management methods, the significantly higher straw yield was evident from the incorporation + consortia+ SSP (M<sub>8</sub>) followed by incorporation + consortia (M<sub>7</sub>) incorporation + SSP (M<sub>5</sub>) and incorporation (M<sub>4</sub>). On the other hand, significantly lower straw yield was observed under removal (M<sub>3</sub>) which was on par with *in-situ* burning (M<sub>1</sub>), retention (M<sub>2</sub>), retention + consortia (M<sub>6</sub>). The straw yield of maize was also comprehensively influenced by the availability of moisture and nutrients due to presence of residues. Higher straw yield was recorded in incorporation + consortia+ SSP (M<sub>8</sub>) might be due to maximum dry matter production and LAI which ultimately enhanced the straw yield. Similar findings were reported by Malhi *et al.* (2006) and Sharma *et al.*, (2012).

The straw yield of maize as influenced by different fertility levels was statistically significant. Among the fertility levels, 125 % RDF recorded higher straw yield. 100 % RDF was the next best treatment. Significantly lower straw yield was observed with 75 % RDF.

The interaction effect was found significant when tested with residue management practices and fertility levels. Among all the tested combinations, M<sub>8</sub>S<sub>3</sub> (incorporation + consortia + SSP with 125 % RDF) recorded significantly higher straw yield but it was statistically at par with that of M<sub>8</sub>S<sub>2</sub> and M<sub>8</sub>S<sub>1</sub> treatment combinations. On the other hand, the significantly lowest straw yield was recorded with the treatment combinations of M<sub>3</sub>S<sub>3</sub> (removal with 125 % RDF) and it was statistically comparable with that of M<sub>1</sub>S<sub>3</sub> (*in-situ* burning with 125 % RDF), M<sub>6</sub>S<sub>3</sub> (retention + consortia with 125 % RDF), M<sub>2</sub>S<sub>3</sub> (retention with 125 % RDF).

### CONCLUSION

Based on the results obtained in the present investigation, it is concluded that under zero till

conditions incorporation + consortia + SSP can be recommended for improving microbial status (bacteria and fungi) and enzyme activity (dehydrogenase) over burning and removal. Further the grain yield and straw yield of maize obtained with the incorporation + consortia + SSP combined with fertility level of 75 % RDF was almost similar with the yield produced with 100 % RDF and 125 % RDF which shows that 25 % of the fertilizers can be saved if the fertilizers are applied along with microbial consortia and SSP in a precise manner.

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## EFFECTIVENESS OF RECOMMENDED CLIMATE SMART AGRICULTURE (CSA) PRACTICES IN TELANGANA STATE

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### ABSTRACT

Climate change has become a global concern demanding attention and action. In a densely populated country like India, particularly the effects of climate change are more detrimental due to its highly vulnerable nature. In view of the increased importance to address the development needs of the farmers, Indian Council of Agricultural Research (ICAR) has launched various projects and programmes to cope with climatic changes by disseminating the climate resilient and smart agricultural technologies. The study was conducted to identify the effectiveness of various recommended Climate Smart Agriculture (CSA) practices to understand the extent to which these technologies are effective and their capability of decreasing the vulnerability of agriculture and subsequent beneficial nature in the long run. The analysis of climatic variability of the Telangana during the study period revealed that the recent decade (2008-2017) has shown the highest variation in annual rainfall (CV= 29.03%) and mean maximum temperature (CV = 2.38 %) compared to the past two decades. The recent decade (2008-2017) has seen a steady increase in the seasonal rainfall across all the months compared to the earlier decade (1998-2007). Ex-post facto research design was followed and 300 respondents selected by using random sampling method. The analysis of effectiveness of CSA practices perceived by the farmers revealed that majority were found to have medium economic efficiency (44.3%) followed by high (34.3%) and low (21.3%) economic efficiency. The possible reason might be attributed due to sub division and fragmentation of the farm size resulting in enhancing of small and marginal land holdings. It may not be feasible to take up all the CSA practices in these holdings. Most of the farmers are not inclined to be in touch with the changes in environmental climate change, this trend may be due to the idiosyncratic behaviour established by the virtue of their medium to old age, less education and possessing low degree of other profile characteristics and majority of them seemed to be not following the Weather Based Agro Advisory Services (WBAAS) regularly as they were taking up the farm activities naturally which limits the effectiveness of CSA practices at critical periods of time.

**Keywords:** Climate Smart Agriculture (CSA), Weather Based Agro Advisory Services (WBAAS), Natural Resource Management (NRM), Climate Resilient Agriculture (CRA), National Innovations in Climate Resilient Agriculture (NICRA)

Climate is continually changing and with projected changes in rainfall patterns and temperature range are expected to affect many biological systems including agriculture. Climate related events such as droughts, cyclones, hailstorm, snowfall, erratic rainfall and fluctuations in temperature negatively contributing to world food production and overall economy. The atmosphere is one of the principal components in determining weather and climate of the earth. Today, climate change has been recognized globally as the most pressing critical issue affecting the mankind survival in the 21<sup>st</sup> century. The average global temperatures are expected to increase by 1.40 - 5.80 degree celsius and this could result in substantial reduction in agricultural yield by the end of the 21<sup>st</sup> century. Increase in temperatures could also result in variation in precipitation pattern and

river flows besides rise in sea level. Quite obviously, countries in the arid and semi-arid regions that are largely dependent on precipitation for their agriculture are likely to be most affected. Erratic and extreme rainfall events could result respectively to, frequent drought and floods. Evidence is mounting to suggest that, in recent years, both frequency and intensity of drought have increased in many parts of the world.

### Climatic Influence on Indian Agriculture

Ample evidences have shown that climate change is not a future threat but a present danger. In view of the extreme climatic uncertainties, it is obvious that Indian agriculture is highly vulnerable to climate change as climate is the direct input for production. Singh (2008) that different estimates by environmentalists indicate location specific uncertain-



ties in the minimum and maximum temperatures which may have adverse impact on agricultural productivity in different agro climatic regions of the country. Such calculations for trends in monthly rainfall may be more during December, January and February in case of West Rajasthan, Punjab, and Haryana. However, there may be slight decrease in October-November rainfall in several locations of the country. The analysis about trends of annual rainfall for the period 1871 to 1999 indicates that there is a shift in the surplus rainfall from western part of country to east.

More than 60 per cent of the total cropped area under irrigation in India is still dependent on the vagaries of monsoon. Studies on climate change have shown that for every 1°C rise in temperature from optimum, yield losses of about 4.6 to 9.4 per cent in rainfed rice (Kumar *et al.*, 2014) and 13 kg/ha in cotton (Raksha, 2014) were recorded. About 11.7 million tonnes of wheat yields and 11 per cent of winter sorghum crop yields were estimated to be lost by 2050 due to climate change and variability (Srivastava *et al.*, 2010). Climate change has projected effects on major crops viz., paddy, sugarcane and groundnut showing decrease in the yields by about 5.2 to 9.5 per cent (Palanisami and Kumar, 2009). Various other factors viz., poor availability of irrigation water, irregularities in the onset of monsoon, heat and cold waves, decline in soil fertility, rise in sea level, saline water intrusion in coastal belts, pests and disease attack, weeds, floods, cyclone and drought tend to cause further losses in the yields. The type of crops to be cultivated would be determined by the climatic variability along with the availability of agricultural inputs like irrigation water, seeds etc.

### **Climate Smart Agriculture (CSA)**

Climate Smart Agriculture concept was originally put forth in 2010 by the UNs Food and Agriculture Organization. Climate smart means agriculture that sustainably increases productivity and resilience to environmental pressures, while at the same time reduces greenhouse gas emissions or removes them from the atmosphere. It is also known as Climate Resilient Agriculture (CRA). CRA means the incorporation of adaptation, mitigation and other practices in agriculture which increases the capacity of the system to respond to various climate

related disturbances by resisting damage and recovering quickly.

CSA is defined by three objectives: firstly, increasing agricultural productivity to support increased incomes, food security and development; secondly, increasing adaptive capacity at multiple levels (from farm to nation); and thirdly, decreasing greenhouse gas emissions and increasing carbon sinks (FAO). What CSA means: - It contributes to achievement of sustainable development goals - It integrates – social, economic and environmental development to meet challenge of providing sustainable (a) livelihood to farmers (b) food security to hungry millions, and (c) eradication of poverty.

It is composed of four pillars: 1. Sustainably increasing agriculture productivity and income 2. Adapting and building resilience to climate change 3. Reducing and/ or removing greenhouse gas emission wherever possible 4. Uses agriculture as a major tool for mitigation of GHG and CO<sub>2</sub> by laying emphasis on its unique capacity to absorb CO<sub>2</sub> and release oxygen through photosynthesis process. It envisages to achieve this through increased cropping, by reducing rain fed areas through integrated water and river basin management and expansion of agriculture on wasteland, wetland, degraded fallow areas and introducing urban agriculture.

CSA encourages agricultural development through approaches that improve food security with low emissions and increase producer incomes. Adoption of CSA practices by farmers has been low globally despite its benefits. Stakeholders must decide the appropriate policies and practices toward a viable agricultural production system.

It is an approach for addressing the development efforts towards the technical, policy and investment condition related issues to achieve sustainable agricultural development for food security under climate change along with eradication of poverty. But its focus is to act at local level where there is already impact of climate.

### **MATERIAL AND METHODS**

The present study confined to an *Ex-post-facto* research design. The state of Telangana was

selected purposively, erstwhile Adilabad, Khammam, Mahabubnagar districts of Telangana state were selected purposively as they classified under the 100 vulnerable districts selected for the NICRA project implementation and subjected to climatic vulnerability across the country. The important climatic vulnerabilities of the districts are high drought proneness, heat stress, mid and terminal dry spells, unseasonal rains etc. Also, average annual rainfall of the district ranges from 750-950 mm. which describes the high vulnerability of the district towards climatic aberrations among the selected districts. Two mandals from each district constituting a total of six mandals were selected for the study. Indervelly, Ichoda mandals of Adilabad district; Wyra, Enkaoor mandals of Khammam district; Hanwada, Jadcharla mandals of Mahabubnagar district were selected. Two villages from each mandal were selected randomly, thus constituting a total of 12 villages for the study. Two villages namely *Anji, Daenapur* of Indervelly mandal, *Narsapur, Gear jam* of Ichoda mandal of the Adilabad district; *Somavaram, Thatipudi* of Wyra mandal, *Nacharam, Emmamnagar* of Enkaoor mandal of Khammam district; *Nainonpally, Ibrahimbad* of Hanwada mandal, *Kodgal, Gangapoor* of Jadcharla mandal of Mahabubnagar district were selected. From each selected village, 25 farmers were selected randomly to comprise a total sample size of 300 for the present study. Taking into consideration the scope and objectives of the study, a well-structured interview schedule was prepared with the help of extensive review of literature and consultation with the experts in the fields of Agricultural Extension, Agronomy, and Statistics.

## RESULTS AND DISCUSSION

### Effectiveness of CSA practices

The results pertaining to the effectiveness of CSA practices as perceived by the farmers are presented

considering various dimensions of the practices viz., economic efficiency, environmental efficiency, and social efficiency.

### I. Economic efficiency of CSA practices as perceived by the farmers

The results in Table 1 indicated that, majority of the farmers were found to have medium (44.3 %) followed by high (34.3 %) and low (21.3 %) economic efficiency. The farmers stated that they were able to enhance their incomes, employment opportunities throughout the year by taking diversified crops, dairy farming, IFS and off farm activities etc. and minimize the aberrations caused due to climate change.

The possible reason might be attributed due to sub division and fragmentation of the farm sizes which is decreasing resulting in more small and marginal land holdings. It may not be feasible to take up all the CSA practices in these holdings. The degree of innovativeness and risk taking also might be minimum for these farmers possessing small holdings, which limits effectiveness of Climate Smart Agriculture practices at critical periods of time.

### II. Environmental efficiency of CSA practices as perceived by the farmers

The results in Table 2 revealed that, majority CSA practices as perceived by farmers were found to have medium (42.3 %) followed by low (41%) and high (16.7 %) environmental efficiency. The possible reason might be attributed due to that farmers are not inclined much to be in touch with the changes in climate change and, this trend may be due to the idiosyncratic behaviour established by the virtue of their medium to old age, less education and possessing low degree of other profile characteristics.

The other reason for the medium to low followed by high levels of perceived environmental efficiency

**Table 1. Distribution of respondents according to the Economic efficiency of CSA practices as perceived by farmers (n=300)**

S.No.	Category	Class Interval	Frequency	Percentage
1.	Low economic efficiency	<5	64	21.3
2.	Medium economic efficiency	6-7	133	44.3
3.	High economic efficiency	>8	103	34.3
	Total		300	100.00

**Table 2. Distribution of respondents according to the Environmental efficiency of CSA practices as perceived by farmers (n=300)**

S.No.	Category	Class Interval	Frequency	Percentage
1.	Low environmental efficiency	<6	123	41
2.	Medium environmental efficiency	7	127	42.3
3.	High environmental efficiency	>8	50	16.7
	Total		300	100.00

of CSA technologies by the farmers may be due to the satisfying results produced by adopting these technologies as a result of which the farmers are slowly realising both short and long lasting effects of these practices, in addition to this the government is giving financial support to take up various NRM activities under watershed. This finding is in conformity with the results of Reddy (2009).

### III. Social efficiency of Climate Smart Agriculture (CSA) practices as perceived by the farmers

The results in Table 3 revealed that, 39 percent of the CSA practices as perceived by farmers were found to have low followed by high (31.7 %) and medium (29.3 %) social efficiency. The probable reason for the low to high followed by medium levels of perceived social efficiency of CSA practices by the farmers may be due to that majority of the respondents had medium levels of adoption of CSA technologies followed by less information seeking behaviour, less social affiliation, environmental awareness, achievement motivation, innovativeness and medium mass media exposure.

### CONCLUSION

The study shows that majority of the respondents were found to have medium economic efficiency followed by high and low levels. The

farmers stated that they were able to enhance their incomes, employment opportunities throughout the year with diversified farming systems and minimize the aberrations caused due to climate change and variability. The major constraint is due to sub division and fragmentation of the farm sizes which is decreasing resulting in more small and marginal land holdings. It may not be feasible to take up all the CSA practices with these holdings. More than one-third of the respondents had perceived environmental efficiency was medium followed by low and high levels. It is due to the satisfying results produced by the technologies that could be attributed to the reason that the farmers are slowly realising both short and long lasting effects of these practices. Majority of the respondents had perceived the CSA practices to have low followed by high and medium social efficiency levels. The farmers were found to be lacking in use of WBAAS, Integrated Pest Management, Site-specific nutrient management and crop insurance, which can be addressed by integration of mass media, information communication technology and other new applications through which the farmers can get information regarding the weather and plan their activities. Also, farmers can make better decisions on market and adopt new technologies. As majority of the farmers belong to semi-medium and small size

**Table 3. Distribution of respondents according to the Social efficiency of CSA practices as perceived by farmers (n=300)**

S.No.	Category	Class Interval	Frequency	Percentage
1.	Low social efficiency	<5	117	39
2.	Medium social efficiency	6	88	29.3
3.	High social efficiency	>7	95	31.7
	Total		300	100.00

land holdings, the CSA technologies developed should be of low cost, user friendly and compatible to increase the rate of adoption. The high levels of achievement motivation, risk taking ability and scientific orientation of the farmers can be best utilized by encouraging them towards taking up various IFS activities, floriculture, poultry, dairy enterprises etc.

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## **PROBLEMS IN SUPPLY CHAIN OF POMEGRANATE (*Punica granatum*) IN ARGHANDAB DISTRICT, KANDAHAR PROVINCE, AFGHANISTAN**

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Pomegranate plant has been grown since ancient times for its delicious fruits and as an ornamental garden for its red, orange or occasionally, creamy yellow flowers. Pomegranate (*Punica granatum L.*) belongs to the Punicaceae family. It is also known as the Chinese Apple or Carthage or Apple with many seeds. About 12 per cent of the total land in Afghanistan is arable and less than 6 per cent currently is cultivated. Agriculture is the backbone of the Afghan economy; according to the statistical book FY (2017-18) the contribution agriculture to the country Gross Domestic Product (GDP) was 20.9 per cent while the labor force engaged in this sector is around 60.8 per cent. The annual growth rate of pomegranate production in Afghanistan was predicted at 2.9 per cent (Fitrat, 2014). Pomegranate contribute of the total population of Afghanistan was about (2) per cent to the total horticultural production in Afghanistan. The local varieties grown in the main production areas of Kandahar province are known for their high quality and productivity. Different varieties of pomegranates are produced in Afghanistan and supplied to the local markets. Through the maturity time of the crop varies according to the climatic conditions, usually the fruit comes into the market during summer and continues into the fall season. The Agriculture sector is entirely run by private enterprise, including farmers, cooperatives, inputs suppliers, herders, agribusiness processors, and exporters. Kandahar province is recognized worldwide for its high quality pomegranate production especially the Kandahar varieties, which are highly preferred by national and international consumers. Of the total land under pomegranate cultivation in the country, Kandahar share is about 36.7 per cent with 39.5 per cent of

the total national production. But due to poor orchard management practices, careless production, widespread pest and diseases, lack of quality inputs and lack of technical and financial support to the farmers both quality and quantity of pomegranate is badly affected and gradually decreasing.

Kandahar is one of the thirty-four provinces of Afghanistan, located in the Southern part of the country next to Pakistan. The climate of Kandahar is that of a true desert. The province receives only (7 to 8 inches) of rain per year, with relative humidity averaging just 38 per cent. The primary data for the study was collected from the respondents by personal interview method using pre-tested schedule. Convenience sampling technique is used for the survey. The sample size was 120 respondents (80 growers and 40 traders). The secondary data was collected from research paper, books, government publication and statistics, journal, provincial department of Agriculture and Ministry of Agriculture, Irrigation and Livestock (MAIL) of Afghanistan.

### **Garrett's Ranking Technique for ranking the problems in the supply chain**

The supply chain of pomegranate for domestic market is 1- production, 2- collection farmer/ traders or cooperative, 3- Kandahar market, 4- Whole seller, 5- Super market or retailer, 6- consumer. The supply chain of pomegranate for foreign market is 1- production, 2- collection farmer/trader, 3- Farmers marketing cooperatives, 4- Foreign supper market/any other market, 5- Foreign consumer. The study of constraints faced by farmers and traders is one of the important objectives of the study. The respondents were asked to rank the problems in production, processing, and

marketing of pomegranate and these ranks were converted into scores by referring to Garretts table. In the study, Garret's ranking technique was used to analyze the constraints of the pomegranate supply chain. The order of the merit given by the respondents was changed into ranks by the using the formula.

$$\text{Percent Position} = \frac{100(R_{ij} - 0.5)}{N_j}$$

Where:

R<sub>ij</sub>=Rank given for i<sup>th</sup> item by j<sup>th</sup> respondent

N<sub>j</sub>= Number of item ranked by i<sup>th</sup> respondent

From the above-mentioned tables, it is indicated that producing pomegranates, farmers faced with many constraints. These constraints/problems are presented in the Table(1) and Table(2).

The list of problems faced by the farmers in the production of pomegranate in the study area was based on the farmers asked to rank the problems in the order of importance. The farmers were asked to rank the problems in order of importance so rated problems were analyzed by using Garrett's ranking technique. The first important problem was found Water scarcity & Drought with a mean score (74.7). Drought which leads to a shortage of water and which again leads to a gradual decrease in the quantity and quality of the produce. Drought is the main problem in Arghandab and Dand districts. The farmers say the underground water level in Arghandab district has significantly plummeted as a result of the prolonged dry spell and water in Karez systems reduced by 75 per cent. The second most important problem was Limited capital or non-availability of financial support a mean score (74.2).

**Table 1. Problems of the supply chain faced by farmers**

Particulars	1	2	3	4	5	6	7	8	9
1- Water scarcity & Drought									
2- Limited capital									
3- Pests & Diseases									
4- Lack of government support									
5- Lack of market									
6- Lack of infrastructure									
7- High cost of Transportation									
8- Lack of quality inputs (Machineries, Pesticides, Fertilizers)									
9- Lack of technical guidance									

**Table 2. Garrett's Ranking Technique for ranking the problems in the supply chain**

No	Problems	Garrett's Mean Score	Rank
1	Water scarcity & Drought	74.7	1
2	Limited capital	74.2	2
3	Pests & Diseases	71.6	3
4	Lack of government support	70.3	4
5	Lack of market	69.5	5
6	Lack of infrastructure	69.3	6
7	High cost of Transportation	67.8	7
8	Lack of quality inputs (Machineries, Pesticides, Fertilizers)	65.4	8
9	Lack of technical guidance	65.3	9

## PROBLEMS IN SUPPLY CHAIN OF POMEGRANATE

Due to this problems, the farmers have to get money from traders in advance in return they sell the pomegranates to, the same traders by very cheap price. Pests & Diseases with mean score of (71.6), Lack of government support with mean score of (70.3), Lack of market with mean score of (69.5), Lack of infrastructure with mean score of (69.3), High cost of Transportation with mean score of (67.8), Lack of quality inputs (Machineries, Pesticides, Fertilizers) with mean score of (65.4) and Lack of technical guidance with mean score of (65.3).

Pomegranate fruit is considered as the suitable fruit for the processing and utilization due to its excellent flavor, color, physic-chemical constitution and therapeutic properties. The pomegranate supply chain management has played important role in the increased consumption and utilization of pomegranate. The most important problem in supply chain of pomegranate in Arghandab district was Water scarcity & Drought followed of Limited capital, Pests & Diseases, Lack of government support, Lack of market, Lack of infrastructure, High cost of Transportation, Lack of quality inputs (Machineries, Pesticides, Fertilizers) and Lack of technical guidance. Therefore, it is important that the government and other responsible bodies should consider the following points.

Association and NGOs need to be more involved by providing technical assistance for pomegranate supply chain management.

Helping the pomegranate growers have access to modern technology to increase quality in production, processing and sorting according to the needs of the international market. It means the government can use both regulatory tools and technological tools to ensure the standardized quality of pomegranates beginning with the first stages.

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## INHERITANCE OF RESISTANCE TO YELLOW MOSAIC VIRUS IN BLACK GRAM (*Vigna mungo* (L.) Hepper)

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Blackgram (*Vigna mungo* (L.) Hepper) also known as uradbean, is one of the important pulse crops of India. India is the largest producer and also consumer of blackgram. It has surely marked itself as the most popular pulse and can be most consequently referred to as the “king of the pulses” due to its delicious taste and numerous other nutritional qualities. Blackgram is superb combination of all nutrients, which contains proteins (25-26 %), carbohydrates (60 %), fat (1.5 %), minerals, amino acids and vitamins. Being a good leguminous crop, it is itself a mini-fertilizer depository, as it has special characteristics of maintaining and restoring soil fertility through fixing atmospheric nitrogen in symbiotic association with Rhizobium bacteria, present in the root nodules. It is short duration pulse crop (Delic *et al.* 2009), usually flowering within 30-60 days of sowing and maturing within 60-90 days.

Among various biotic and abiotic yield limiting factors, mungbean yellow mosaic disease (MYD) caused by mungbean yellow mosaic virus (MYMV) is the most destructive limiting factor in blackgram. Infection of MYMV may cause up to 85-100 % yield loss in uradbean depending on the stage and severity of infection (Singh *et al.*, 1980). Symptoms include initially mild scattered yellow spots appear on young leaves and trifoliolate leaves emerging from the growing apex show irregular yellow and green patches alternating with each other, spots gradually increase in size and ultimately some leaves turn completely yellow. Diseased plants are stunted, mature late and produce very few flowers and pods.

The virus is transmitted by white flies (*Bemisia tabaci*). Management of MYMV is often linked with control of the *Bemisia tabaci* population by spraying insecticides, which is sometimes ineffective because of high population pressure. The chemical management of the vector is expensive since numerous sprays of the insecticides are required to control the whitefly. Spraying often also lead to health hazards and ecological effluence. On the contrary, use of virus resistance varieties is the most economical, efficient and environmental friendly approach.

The awareness on mode of inheritance of YMV resistance is crucial to develop relevant breeding strategy targeted to incorporation useful gene contributing resistance to MYMIV. There are only few divergent reports were published on mode of inheritance and gene governing the resistance. In blackgram, monogenic dominant nature of resistance was reported by Gupta *et al.*, (2005) while it was noted to be digenic recessive by Verma and Singh (1986). The chief objective of this present study was to analyse gene action involved in the inheritance of MYMV resistance in black gram in segregating F<sub>2</sub> populations.

To study the inheritance pattern of mungbean yellow mosaic virus resistance, F<sub>2</sub> mapping population was developed from the cross between the susceptible variety MBG 207 as female parent and resistant variety PU 31 as a pollen parent. Sowing of parent material and crossing programme were performed during *kharif* 2017-18 season under shade net at Institute of Biotechnology (IBT), PJTSAU,



## INHERITANCE OF RESISTANCE TO YELLOW MOSAIC VIRUS IN BLACK GRAM

Rajendranagar, Hyderabad. F<sub>1</sub> seeds are collected and raised to develop segregating F<sub>2</sub> population during *rabi* 2017-18 season at college farm. The parental lines and 177 F<sub>2</sub> populations were screened for MYMV reaction during *rabi* 2018-19 season under natural hot spot condition at ARS Madhira, Khammam to analyse the pattern of MYMV resistance inheritance.

An infector row technique was used for evaluating parent's and F<sub>2</sub> generation for MYMV resistance. After every ten rows, one row of highly susceptible genotype MBG 207 was grown as an infector. The MYMV occurrence was recorded on all the plants of F<sub>2</sub> population of the cross based on the visual scores. The rating scale implied by Bashir *et al.*, (2005) is given below at Table 1. To confirm goodness of fit of the performed cross Mendelian segregation ratio for MYMV (resistance: susceptible) in the segregating population was tested through chi-square test.

To deduce the inheritance pattern of MYMV resistance, blackgram cross MBG 207 × PU31 were

evaluated and Chi-square test was developed to confirm the expected deviation from the Mendelian segregation ratio of segregating generation F<sub>2</sub> and the results are presented in Table 2. All the accessible information pertaining to MYMV resistance confirmed that the F<sub>1</sub>s of the cross were shown symptoms to MYMV and this marked clearly that the resistance was susceptible over dominance. With respect to observed: expected F<sub>2</sub> segregation ratio for resistance: susceptible chi-square test showed non-significance chi-square value confirmed that the expected ratio, fitted well with 1:3 (resistance : susceptible) in F<sub>2</sub>. It indicates a typical monogenic recessive gene is governing resistance and susceptibility reaction against MYMV in blackgram.

There are many reports about the genetics of resistance to MYMV disease, monogenic recessive control of yellow mosaic resistance was reported by authors Pal *et al.*, (1991), tri-genically controlled with inhibitory gene action in four cross combinations

**Table 1. Grouping of 177 F<sub>2</sub> population of the cross MBG-207 x PU-31 based on MYMV reaction under field condition (Bashir *et al.*, 2005).**

Scale	% Infection category	Infection group	Reaction	No of lines	Final classification of F <sub>2</sub> population on the basis of YMV infection
0	All plants free of virus symptoms	Highly Resistant	HR	10	52
1	1-10 % infection	Resistant	R	25	
2	11-20 % infection	Moderately Resistant	MR	17	
3	21-30 % infection	Moderately Susceptible	MS	20	125
4	30-50 % infection	Susceptible	S	66	
5	More than 50 %	Highly Susceptible	HS	39	

**Table 2. Chi-square test for segregation of disease resistant reaction in F<sub>2</sub> population.**

F <sub>2</sub>	Total plants	Disease Resistant Reaction				d. f.	Expected ratio (R:S)	x <sup>2</sup> calculated value	x <sup>2</sup> table value
		Observed		Expected					
		Susceptible	Resistant	Susceptible	Resistant				
MBG 207 X PU 31	177	52	125	44.25	132.75	1	1:3	1.809 <sup>NS</sup>	3.841

observations by Vadivel *et al.*, (2021), monogenic dominant nature of resistance was reported by Dahiya *et al.*, (1977), Kaushal and Singh (1988) and Gupta *et al.*, (2005) and while it was reported to be digenic recessive by Singh (1980), Dwivedi and Singh (1985) and Verma and Singh (1986).

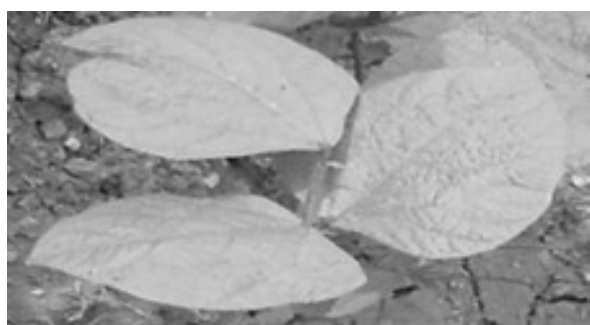
The ratio fitted well with different ratios of mendelian in F<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub> populations of five selected cross combinations revealed the nature of inheritance as duplicate, complementary and inhibitory by Durgaprasad *et al.*, (2014). Single dominant gene governing inheritance was conformed in blackgram cultivar “VBN (Bg) 4” by Vinoth *et al.*, (2014). The involvement of complementary gene action, interaction of duplicate dominant and duplicate recessive types of epistasis interactions for MYMV

resistance inheritance was noted in different selected crosses by Thamodhran *et al.*, (2016).

The phenotypic data based on resistance and susceptibility reactions to the disease caused by YMV, segregated in a 1:3 (R:S) ratio in the F<sub>2</sub> population. Therefore, the resistance to YMV is likely to be controlled by a recessive gene in Blackgram. Thus this information would pave the way for YMV resistance breeding and mapping of the gene with linked molecular markers.

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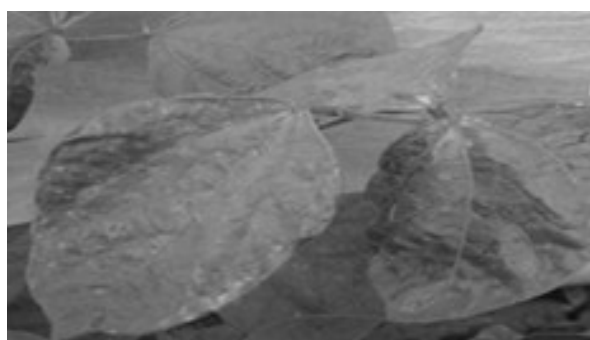
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HIGHLY RESISTANT-0



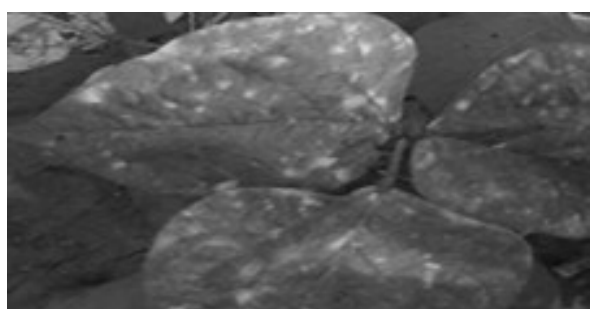
SUSCEPTIBLE-3



RESISTANT-1



MODERATELY SUSCEPTIBLE -4



MODERATELY RESISTANT-2



HIGHLY SUSCEPTIBLE-5

Plate 1. Screening of segregating material for YMV disease reaction.

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