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Biochemical characterization and genotypic identification in hybrid maize - Hema

S P Kiran and **B C Chennakeshava**
Department of Seed Science and Technology, College of Agriculture, UAS, GKVK, Bengaluru

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ABSTRACT

The study was conducted at V.C. Farm, MARS, Mandya during the rainy seasons of 2015 and 2016 to characterize and identify hybrid maize genotypes (variety: Hema) using biochemical approaches. Twenty-eight genotypes were evaluated through colour-based biochemical tests. In the NaOH test, 12 genotypes showed light yellow, 8 dark yellow, and the rest no reaction. The KOH test indicated 16 light yellow, 9 medium yellow, 1 dark yellow, and the remaining as absent. The standard phenol test classified 4 genotypes as brown, 14 light brown, 1 dark brown, and 9 as absent. The modified phenol test with 1% $CuSO_4$ showed 4 brown, 13 light brown, 2 dark brown, 8 light yellow, and 1 black genotype. Electrical conductivity was highest in MAI-143 and MAI-33174 (341.20 and 337.50 µS ppm^{-1}) and lowest in MAI-267 (188.59 µS ppm^{-1}). MAI-267 recorded the highest TDH activity (1.77), soluble protein (364.45 µg), and sugar content (21.95%), while MAI-143, MAI-204, MAI-194, and MAI-12 showed the lowest values. These results highlight significant biochemical variation among genotypes, supporting their use in hybrid identification and maize improvement programs.

KEY WORDS: *Maize-Hema*; *Seed Treatments*; *Packaging* Materials; Seed *Storability*

1. Introduction

Maize (*Zea mays* L.) is one of the most important staple food crops globally, ranking third after wheat and rice. Its significance lies not only in its high production potential but also in its adaptability to a wide range of agro-climatic conditions. In India, maize plays a crucial role in the national economy, comparable to other major cereals such as rice, wheat, and millets. Apart from being a vital food source for humans, maize serves as a major component in animal feed and is widely used in industrial applications for producing starch, syrup, alcohol, acetic acid, lactic acid, and other derivatives. The quality of seed is a critical factor in ensuring successful crop establishment and productivity, particularly in maize. On-farm seed treatment techniques have been promoted among small-scale farmers to improve germination and early plant vigor, as treated seeds can absorb water more efficiently under field conditions, leading to faster and more uniform germination (Harris *et al*., 2007).

Traditional varietal characterization based on morphological traits, while useful, presents several limitations including environmental influence, seasonal dependency, space requirements, and time-consuming procedures. To overcome these challenges and accelerate the

CONTACT S P Kiran kiransp7@gmail.com

identification process, biochemical tests have emerged as efficient alternatives. These tests, including phenol reaction, modified phenol test, and alkali tests using NaOH and KOH, are rapid and effective for identifying genotypes based on colour changes in seeds and solutions upon chemical exposure. Such biochemical markers have proven valuable in detecting varietal mixtures and classifying a large number of genotypes into distinct groups, as demonstrated in crops like greengram (Chakrabarthy and Agrawal, 1990). Therefore, incorporating biochemical approaches offers a reliable and practical method for varietal characterization and purity assessment, especially in hybrid maize breeding programs.

2. Material and Methods

The study was conducted at the Zonal Agricultural Research Station (ZARS), V.C. Farm, Mandya, under the University of Agricultural Sciences (UAS), Bengaluru, during the rainy seasons of 2015 and 2016. A total of twenty-eight hybrid maize genotypes were obtained from the All India Coordinated Research Project (AICRP) on maize. The seeds were cleaned, dried to a safe moisture content of 10%, graded to uniform size, and used for characterization studies. Each genotype was sown in 4-meter-long rows with a spacing of 30 × 60 cm, and standard agronomic and plant protection practices were followed throughout the crop season. Biochemical characterization of the genotypes was performed using various standard tests, as detailed below:

Sodium Hydroxide (NaOH) Test

Seeds were soaked in 2% NaOH solution for two hours, and the resulting colour change in the solution was observed as per the method of Papp *et al*. (1997). Based on the intensity of colour, genotypes were categorized into three groups: light yellow, dark yellow, and dark red.

Potassium Hydroxide (KOH) test

Seeds were soaked in 4% KOH solution for two hours, and colour changes were recorded following the same reference method. Genotypes were grouped into four categories: light yellow, medium yellow, medium red, and dark red.

Standard phenol test

Seeds were pre-soaked in distilled water for 24 hours and then placed on filter paper moistened with 1% phenol solution. After 24 hours, seed coat colour changes were recorded and genotypes were classified into four groups: absent, light brown, brown, and dark brown.

Modified phenol test

Seeds were soaked in 0.5% $CuSO_4$ solution for 24 hours and then transferred to filter paper saturated with 1% phenol solution. After 24 hours, colour reactions were noted, and genotypes were categorized as light yellow, brown, dark brown, or black.

Electrical conductivity of seed leachate ($\mu S\ ppm^{-1}$)

The electrical conductivity (EC) of seed leachate was measured according to ISTA (2010). Twenty-five seeds per genotype, with three replications, were soaked in 50 ml of distilled water for 18 hours at 25 ± 1°C. After incubation, the leachate was decanted, and EC was measured using a digital conductivity meter (Model D1 9009) and expressed in $\mu S\ ppm^{-1}$.

Total Dehydrogenase (TDH) activity (A480 nm)

TDH activity was estimated as per Perl *et al*. (1978). Ten randomly selected seeds from each of three replications were pre-soaked in water for 24 hours. The seeds were then bisected longitudinally and soaked in 0.5% tetrazolium chloride solution, followed by incubation at 25 ± 1°C in the dark for six hours. After thorough washing, the red formazan pigment formed in the embryos was extracted with 5 ml of 2-methoxy ethanol over 6–8 hours in airtight containers. The extract's absorbance was read at 480 nm using a spectrophotometer (Model Mini Spec 17), and TDH activity was expressed in absorbance units.

3. Results and Discussion

The biochemical characterization of twenty-eight maize genotypes was conducted using a series of rapid chemical tests to distinguish genotypic variability. The sodium hydroxide (NaOH) test categorized the genotypes into three distinct groups based on seed colour reaction. Among the genotypes, twelve (MAI-728, MAI-137, MAI-316, MAI-32575, MAI-33174, MAI-44671, MAI-202, MAI-143, MAI-211, MAI-194, MAI-13, and MAI-19) exhibited a light yellow reaction, while eight genotypes (MAI-280, MAI-105, MAI-215, MAI-144, MAI-214, MAI-155, MAI-283, and MAI-256) showed dark yellow (Table 1). The remaining genotypes were grouped under the absent category. The observed colour variation is attributed to the interaction of seed constituents, particularly secondary metabolites, with the alkaline medium, reflecting underlying genetic differences in enzyme systems (Chakrabarthy & Agrawal, 1990; Anithalakshmi, 2002; Thangavel *et al*., 2005).

In the potassium hydroxide (KOH) test, genotypes were grouped into four categories: light yellow, medium yellow, dark yellow, and absent. Sixteen genotypes, including MAI-135, MAI-728, MAI-137, MAI-267, MAI-211, MAI-204, MAI-44671, MAI-10835, MAI-202, MAI-12, MAI-194, MAI-13, MAI-256, MAI-19, MAI-316, and MAI-264, showed a light yellow reaction. Nine genotypes (MAI-280, MAI-105, MAI-32575, MAI-215, MAI-33174, MAI-144, MAI-214, MAI-143, and MAI-155) exhibited medium yellow, while MAI-283 alone showed dark yellow coloration. The rest were placed in the absent category. These reactions are indicative of seed chemical composition and enzymatic activity, which are genetically controlled, consistent with findings in wheat, sorghum, and rice (Dileepkumar *et al*., 2015).

The standard phenol test revealed phenol-based oxidative reactions on the seed coat, which were used to group genotypes. Four genotypes (MAI-135, MAI-33174, MAI-729, and MAI-194) showed a brown reaction; fourteen genotypes showed light brown (including MAI-728, MAI-105, MAI-267, MAI-316, MAI-32575, MAI-211, MAI-215, MAI-204, MAI-144, MAI-10835, MAI-143, MAI-283, MAI-256, and MAI-19), while MAI-298 alone showed a dark brown reaction. The remaining nine genotypes exhibited no response. The phenol reaction serves as a marker for polyphenol oxidase activity, an established trait used in varietal identification (Joshi & Banerjee, 1970), with similar observations reported in sorghum and maize (Thangavel *et al*., 2005).

In the modified phenol test with 1% $CuSO_4$, which enhances enzymatic reactions due to catalytic

action of Cu^{2+} ions, the genotypes were grouped into five categories: brown, dark brown, light brown, light yellow, and black. Four genotypes (MAI-316, MAI-10835, MAI-280, and MAI-298) showed brown coloration; thirteen (including MAI-135, MAI-728, MAI-202, MAI-33174, MAI-44671, MAI-144, MAI-214, MAI-267, MAI-194, MAI-729, MAI-283, MAI-256, and MAI-19) were classified as light brown; two genotypes (MAI-32575 and MAI-211) were dark brown; eight (MAI-137, MAI-105, MAI-264, MAI-204, MAI-143, MAI-12, MAI-13, and MAI-155) exhibited a light yellow response, and MAI-215 alone showed a black reaction. These results

Table 1: Response of maize genotypes to different rapid chemical tests

Maize genotypes	Phenol	Modified phenol	NaOH	KOH
MAI-135	Brown	Lightbrown	Absent	Lightyellow
MAI-728	Lightbrown	Lightbrown	Lightyellow	Lightyellow
MAI-137	Absent	Lightyellow	Lightyellow	Lightyellow
MAI-105	Lightbrown	Lightyellow	Darkyellow	Mediumyellow
MAI-267	Lightbrown	Lightbrown	Absent	Lightyellow
MAI-316	Lightbrown	Brown	Lightyellow	Lightyellow
MAI-32575	Lightbrown	Darkbrown	Lightyellow	Mediumyellow
MAI-264	Absent	Lightyellow	Absent	Lightyellow
MAI-211	Lightbrown	DarkBrown	Lightyellow	Lightyellow
MAI-215	Lightbrown	Black	Darkyellow	Mediumyellow
MAI-33174	Brown	Lightbrown	Lightyellow	Mediumyellow
MAI-204	Lightbrown	Lightyellow	Absent	Lightyellow
MAI-44671	Absent	Lightbrown	Lightyellow	Lightyellow
MAI-144	Lightbrown	Lightbrown	Darkyellow	Mediumyellow
MAI-10835	Lightbrown	Brown	Absent	Lightyellow
MAI-214	Absent	Lightbrown	Darkyellow	Mediumyellow
MAI-202	Absent	LightBrown	Lightyellow	Lightyellow
MAI-143	Lightbrown	Lightyellow	Lightyellow	Mediumyellow
MAI-280	Absent	Brown	Darkyellow	Mediumyellow
MAI-12	Absent	Lightyellow	Absent	Lightyellow
MAI-194	Brown	Lightbrown	Lightyellow	Lightyellow
MAI-298	Darkbrown	Brown	Absent	Absent
MAI-729	Brown	Lightbrown	Absent	Absent
MAI-13	Absent	Lightyellow	Lightyellow	Lightyellow
MAI-155	Absent	Lightyellow	Darkyellow	Mediumyellow
MAI-283	Lightbrown	Lightbrown	Darkyellow	Darkyellow
MAI-256	Lightbrown	Lightbrown	Darkyellow	Lightyellow
MAI-19	Lightbrown	Lightbrown	Lightyellow	Lightyellow

align with earlier findings that Cu^{2+} enhances phenol oxidation and seed coat colour changes (Banerjee & Chandra, 1977; Dileepkumar *et al*., 2015).

Significant differences were also observed in physiological parameters (Table 2). The electrical conductivity (EC) of seed leachate, an indicator of membrane integrity, was lowest in MAI-267 (188.59 µS ppm^{-1}), followed by MAI-280 (198.20 µS ppm^{-1}), indicating superior membrane stability. The highest EC was recorded in MAI-143 and

Table 2: Variation in EC (µSppm^{-1}), TDH activity (A_{480} nm), Total soluble protein (µg g^{-1} of seed) and Total soluble sugars (%) in different maize genotypes

Maize genotypes	Electrical conductivity of seed leachate (µSppm^{-1})	TDH activity (A_{480})	Total soluble protein (µg g^{-1}ofseed)	Total soluble sugars (%)
MAI-135	218.20	0.61	262.17	19.10
MAI-728	323.40	0.82	292.28	19.06
MAI-137	287.10	1.05	319.96	19.78
MAI-280	198.20	1.31	333.08	19.50
MAI-105	231.87	1.06	317.32	18.68
MAI-267	188.59	1.77	364.45	21.95
MAI-316	244.80	1.38	338.23	19.46
MAI-32575	288.40	0.92	281.27	19.25
MAI-264	257.00	0.53	253.86	18.45
MAI-211	295.20	0.74	223.58	17.73
MAI-215	270.10	1.26	319.71	19.96
MAI-33174	337.50	0.45	285.82	18.92
MAI-204	299.10	0.50	242.21	17.82
MAI-44671	275.40	0.87	315.19	21.32
MAI-144	262.00	1.63	348.52	19.86
MAI-10835	239.57	1.57	255.54	18.36
MAI-214	298.00	1.09	328.19	19.93
MAI-202	219.70	0.81	251.63	18.54
MAI-143	341.20	0.41	213.09	18.08
MAI-12	277.50	0.83	241.89	17.29
MAI-194	201.20	1.25	316.13	17.18
MAI-298	244.40	0.78	291.93	19.00
MAI-729	248.20	1.35	326.15	20.33
MAI-13	260.70	1.08	313.15	20.38
MAI-155	252.20	1.12	342.28	21.43
MAI-283	244.50	1.57	362.19	20.82
MAI-256	245.20	1.17	289.03	18.36
MAI-19	251.70	1.18	306.19	20.31
Mean	260.75	1.04	297.68	19.32
SEm±	4.22	0.03	5.28	0.51
CD(P=0.05)	15.85	0.11	19.81	1.91
CV(%)	2.84	4.85	3.09	4.59

MAI-33174 (341.20 and 337.50 µS ppm^{-1}), suggesting greater seed deterioration. These findings reflect differences in seed quality and viability.

The total dehydrogenase (TDH) activity, a measure of metabolic activity, also varied significantly. MAI-267 recorded the highest activity (1.77 A_{480} nm), while the lowest was observed in MAI-143 and MAI-204 (0.41 and 0.50, respectively). This suggests a higher respiratory and metabolic efficiency in MAI-267, possibly due to a more robust enzyme system.

Regarding biochemical constituents, MAI-267 again recorded the highest soluble protein content (364.45 µg), while MAI-143 had the lowest (213.09 µg). In terms of soluble sugar content, MAI-267 was superior (21.95%), whereas MAI-194 and MAI-12 recorded the lowest levels (17.18% and 17.29%, respectively). These differences reflect genotypic variation in storage reserves and metabolic potential. Notably, genotypes such as MAI-267, MAI-155, MAI-283, MAI-144, MAI-729, MAI-280, and MAI-215 demonstrated both high protein and sugar content, making them promising candidates for future breeding programs aimed at improving seed quality traits.

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Effect Performance of Cotton in Maharashtra: A Long-Term Analysis

Kaveri Khairnar[1], **Sachin More**[2] and **Ranjit Chavan**[2]
[1]Junior Research Assistant, Department of Agricultural Economics, Mahatma Phule Krushi Vidyapeeth, Rahuri. Maharashtra, India.
[2]Associate Professor, Department of Agricultural Economics, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

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ABSTRACT

The growth and instability in the area, production and yield of cotton in Maharashtra was analysed for pre liberalisation and post liberalisation period. The growth behaviour of area, production and productivity of cotton was studied for sixty years (1960-60 to 2019-20). Compound growth rates were estimated with the help of exponential growth model. However, instability in the area, production and yield of cotton was worked out by estimating Cuddy Della Valle instability index. The results revealed that the area, production and productivity of the cotton increased significantly after the post liberalisation period. A significant increase in production and productivity of cotton was noticed in the state after adoption of Bt cotton varieties. Yield improvement was the primary reason behind the phenomenal growth in the cotton production. Second sub-period of the study showed more fluctuations in production and productivity of cotton. Area of cotton was found to be steadier than production and productivity. Though, adoption of Bt hybrids enhanced the cotton productivity in the state but, it was associated with moderate instability.

KEY WORDS: *Cotton*; *Cuddy-Della Valle Index*; *Growth*; *Instability*; *Performance*

1. Introduction

Cotton, the leading cash crop in India plays a significant role in the Indian agriculture. Many rural families and smallholder farmers in the country depend on the cotton to sustain their livelihood (Hazell, 1982; Della, 1979). It provides basic raw material to the textile industry. Cotton is referred as the backbone of the textile industry. It contributes significantly to agriculture and industry in terms of farm income, employment and export earnings. Cotton provides valuable by-products like lint, oil, seed meal, hulls and biomass (Chand and Raju, 2008). It has huge demand from the industry side, and hence it is also called as White Gold (Mehra, 1981).

By occupying significant contribution in area (120.69 lakh Hectares) and production (362.18 lakh bales), India became the largest producer of cotton in the world. However, India occupied 38th rank with a productivity of 510 kg per hectare during 2021-22. In the case of cotton export, India occupied the third position with 5.5 million bales. Maharashtra, Gujarat, Andhra Pradesh, and Telangana are the important cotton growing states in India. These states are collectively known as the

CONTACT Kaveri Khairnar kaveriyk@gmail.com

Cotton Basket of India due to its significant share in the cotton production.

The state of Maharashtra has 32 per cent area under cotton (39.37 lakh ha) and contributes around 24 per cent of its production (89.86 lakh bales) in the country. Hence, the long-term performance of the cotton crop in Maharashtra state was studied by estimating the compound growth rate and instability in the area, production and productivity of cotton at the state level.

2. Material and Methods

The present study was carried out in Maharashtra state. It was based on secondary data collected from various issues of Statistical Abstracts of Maharashtra State (Plate 1) published by Directorate of Economics and Statistics, Maharashtra and online data bank of Economic and Political Weekly and International Crop Research Institute for Semi-Arid Tropics. Data were collected for the period of sixty years from 1960-1961 to 2019-2020. The study period was further categorized into two sub-periods; the first period represents the pre liberalization period **(**1960-61 to 1989-90) and the second period represents the post liberalization period (1990-91 to 2019-20).

2.1 Growth rates

The compund growth rates were estimated with the help of exponential model assuming additive error terms.

$$Y_t = \text{constant} * (1+CGR)^t + e_t$$

Where,

Y_t is the time series data for area / production / yield for year t,

t is the time trends for years of interest,

e_t is the error term and

CGR is the compound growth rate for the period under consideration.

The data were smoothened with the help of three-year central moving average techniques to remove bias from the data induced by the outliers (Sawant, 1983).

2.2 Instability

Instability in area, production and productivty of the cotton was studied by estimating Cuddy Della Valle Index. It is a modification of the coefficient of variation and found superior over other methods (Cuddy and Valle, 1978). It is calculated with the given formula:

$$I_x = \frac{SEE}{\bar{y}} * 100$$

Where,

I_x = Instability index

SEE = Standard error of the trend line estimates

$\bar{y}$ = Average value of the time series data

The growth and instability in area, production and productivty of the cotton was estimated by using Statistical Analysis System (SAS) software.

Plate 1 Map of Maharastra State

3. Results and Discussion

The long-term performance of the cotton in Maharashtra was evaluated with the help of two variables *i.e*. growth and instability in the area, production and productivity of cotton.

3.1 Growth rates

Table 1 and Fig. 1 presented the mean value and compound growth rates of the area, production and yield of cotton in Maharashtra. The area of cotton increased from 2580.94 thousand hectares to 3382.64 thousand hectares during two sub-periods, respectively in the state. However, the area of cotton showed an expansion of 2981.79 thousand hectares in the entire study period. In the case of production, it was 1216.62 thousand bales during the first period, which was further increased to 4280.06 thousand bales during the second period. The average cotton production was 2748.34 thousand bales during the overall period. Average productivity of cotton was improved after the adoption of Bt Hybrids cotton. Average yield of cotton was raised to 205.90 kg per hectare during the second period from initial yield of 80.30 kg per hectare in during the first period. During the overall period of the study, the yield of cotton was 143.1 kg per hectares. Thus, the second period revealed enhancement in the area, production and yield of cotton in Maharashtra improved over the first period.

The area of cotton showed non-significant growth in the first period while it grew @ 1.91 per cent and 0.96 per cent in the second and the overall period, respectively. The growth in the second and the overall period was significant by 1 per cent level of significance. Small farmers of the Maharashtra preferred the cotton crop in their cropping pattern due to their profitability over another competitive crop. It was the primary reason behind expansion in the cotton area.

Likewise, cotton production showed significant growth in production as it registered growth rate of 1.40 per cent and 5.32 per cent in the first and second periods. The significant growth in the cotton production by 1.40 per cent was mainly due to significant productivity improvement by 1.43 per cent in the first period as area growth was non-significant. However, the cotton production grew significantly by 5.32 and 4.86 per cent per annum, respectively during the second and the overall period. Improvement in productivity of cotton by 3.45 per cent per annum and area expansion by 1.91 per cent per annum in the second period led to significant positive growth of the cotton production in the state.

Table 1: Estimates of the compound growth rate of area, production and yield of cotton in Maharashtra

Variables	Period-I		Period-II		The overall Period	
	Mean	C.G.R	Mean	C.G.R	Mean	C.G.R
Area	2580.94	-0.09^{NS}	3382.64	1.91**	2981.79	0.96**
Production	1216.62	1.40**	4280.06	5.32**	2748.34	4.86**
Yield	80.30	1.43**	205.90	3.45**	143.1	3.26**

Note : Area in ‘000’ ha; Production in '000' bales of 170 kg each ; Yield in kg/ha
*significant at 5 per cent **significant at 1 per cent

Yield improvement of the cotton by 3.26 per cent was the significant contributing factor as compared to area (0.96 per cent) during the entire study period. Average yield of cotton in Maharashtra during the first period increased by 1.43 per cent and by 3.45 per cent during the second period.

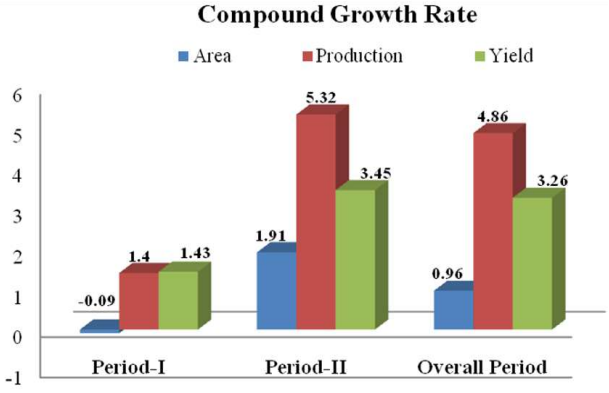

Fig. 1: Compound growth rates of area, production and yield of cotton Instability

3.2 Instability

Table 2 and Fig. 2 presented the estimates of instability in area, production and yield of the cotton crop in Maharashtra state. Instability analysis showed that second sub-period of study revealed higher fluctuations in the area, production and productivity of the cotton as compared to the first sub-period. The yield of the cotton crop was more unstable compared to its area in both the sub-periods as well as during the entire study period. Instability in production and productivity of cotton was increased during the second sub-period of the study compared to the first sub-period. It was increased to 34.76 per cent from 27.05 per cent per annum in production. In regards to yield, instability was increased from 26.22 to 31.84 per cent per annum.

The values of CDV for area, production and productivity of cotton were 12.61, 49.86 and 37.45 per cent per annum, respectively during the entire study period. Production is an interaction of area and yield, so instability in yield and area was reflected in the production series. Thus, instability in cotton production was maximum compared to yield and area at the state level.

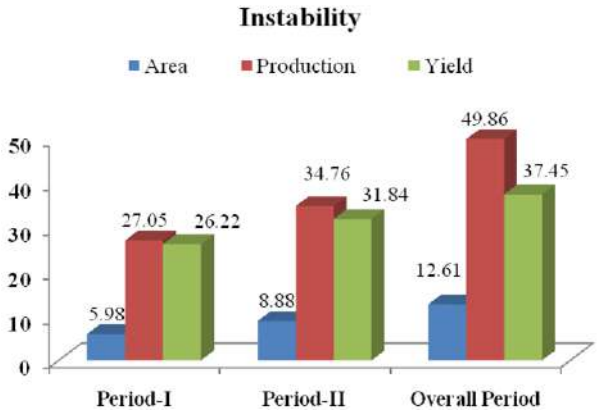

Fig. 2: Instability index of area, production and yield of cotton

Table 2: Estimates of instability index of area, production and yield of cotton in Maharashtra

Variables	Period-I		Period-II		The overall Period	
	Mean	CDV	Mean	CDV	Mean	CDV
Area	2580.94	5.98	3382.64	8.88	2981.79	12.61
Production	1216.62	27.05	4280.06	34.76	2748.34	49.86
Yield	80.3	26.22	205.9	31.84	143.1	37.45

Note: Period I: 1960-61 to 1989-90, Period II: 1990-91 to 2019-20, The overall period: 1960-61 to 2019-20

4. Conclusion

The adoption of Bt hybrids technology led to significant expansion in the area, production and productivity of cotton in Maharashtra state. A significant growth in cotton production was accompanied with moderate instability (Ray, 1983). Bt cotton hybrids improved the cotton yield but, it also resulted in fluctuations in cotton yield. The significant growth in cotton production was mainly due to significant productivity improvement and area expansion.

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Multivariate analysis and multi-trait index based selection of Maize (*Zea mays* L.) inbreds for agromorphological and yield components

Kumari Vinodhana N[1], **Sivakumar S**[2] and **Himakara Datta M**[2]

[1] Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.
[2] Department of Genetics and Plant Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

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ABSTRACT

Germplasm is pivotal in breeding, significantly impacting targeted efforts through genetic diversity. Assessing genetic parameters aids researchers in precise germplasm selection for enhanced gains. Ideotype-based breeding refines this by tailoring plant types to specific goals. The Multi Trait Genotype Ideotype Distance Index (MGIDI) defines ideotype parameters and aids in selecting genotypes closely aligned with desired traits. A study conducted at Tamil Nadu Agricultural University during 2022-23 utilized 55 maize germplasm lines in a randomized block design, evaluating nine biometrical traits. Analysis of Variance revealed significant genotype differences for all traits, with grain yield positively associated with plant height, cob length, cob diameter, kernel row number, and kernels per row. Principal component analysis identified three PC with eigen value >1 explaining 74.44% cumulative variance, associating the first component with yield and cob traits, the second with flowering traits, and the third PC with shelling percentage and plant height. Two ideotypes based on maturity i.e. medium and late maturity were defined and MGIDI was calculated. Selection gains were positive for all the traits, and Genotype B. NO 1265-6-2 (G8) was ranked as the closest to the medium maturing ideotype. Similarly, Genotype UMI 1003-2-3 (G10) was closest in resemblance to the late maturing ideotype and the selection gains were positive for all the traits except plant height. This study emphasizes the critical role of germplasm selection and ideotype-based breeding in enhancing genetic gains in maize breeding programs.

KEY WORDS: *Variability*; *Correlation*; *Ideotype*; *MGIDI*; *Selection gain*

1. Introduction

Maize, often known as corn, is a key grain crop in the Poaceae family that has enormous worldwide agricultural relevance (Shikha *et al*., 2021). Its origin may be traced back to the Tehuacan Valley in Mexico, where it was first domesticated. Maize has a genome size ranging from 2.4 to 2.7 gigabase pairs (Gbp) and is notable for being one of the first plant genomes of gigabase size to be sequenced using novel approaches such as omics technology (Rabinowicz and Bennetzen, 2006). This crop is critical for producing food, animal feed, and low-cholesterol edible oil for human and cattle use. Its domestication required considerable morphological changes, allowing the plant to adapt from its tropical origins to survive in a variety of environmental situations (Gálvez, 2020).

Maize agriculture in India covers around 10 million hectares, yielding 34.3 million metric

CONTACT Kumari Vinodhana soundhini@yahoo.com

tonnes and contributing just 2% to world production in the 2022-2023 timeframe (US-NAAS, 2023). Accurately estimating and projecting grain output is critical for guaranteeing food security and developing effective food policy (Ren *et al*., 2023). Understanding the relationship between yield and its constituent parts is essential for increasing agricultural productivity through strategic breeding (Datta *et al*., 2023). To precisely gauge the level of genetic diversity within a population, genotypic coefficients of variation (GCV), phenotypic coefficients of variation (PCV), broad-sense heritability (h2b), and genetic advance (GA) must be meticulously measured. Efficient selection methods rely on a parent population with a high degree of diversity. Metrics such as PCV and GCV give information on the amount of variation in a population, whereas heritability represents the proportion of a trait that is passed down to future generations (Adhikari *et al*., 2018). Understanding heritability helps guide selection approaches, forecast improvements, and evaluate the significance of genetic influences (Hadi and Hassan, 2021). The level of development observed in a certain characteristic under specific selection forces is measured by genetic advance. Higher genetic progress combined with enhanced heritability gives ideal circumstances for selection operations.

Grain yield in maize is a complex quantitative trait; hence direct selection for this trait is not fruitful. The correlation analysis points to the interrelationships between the yield and other traits taken under study. The information on these interrelationships can be utilized to structure a selection strategy to improve the yield through the selection of yield associated traits (Pavlov *et al*., 2015). The Principal Component Analysis (PCA) is a dimensionality reduction technique which is useful for inferring or identifying the patterns amongst the germplasm and the traits responsible for the variation (Guei *et al*., 2005). It can be used for genetic improvement of important traits contributing to the variability (Das *et al*., 2017).

An Ideotype can be defined as a plant type that has a combination of all the ideal traits (Rocha *et al*., 2018). The complexity of the selection of a superior genotype with all the favourable traits can be attributed to the quantitative nature of inheritance of those traits. Several selection indices have been proposed for selecting superior genotypes based on the defined selection criteria (Céron-Rojas and Crossa, 2018). The limitation of these indices is the effective conversion of the economic value of these traits into weightage for selection of genotypes. This roadblock was overcome by (Olivoto and Nardino, 2020) through the development of a multi trait selection index called the Multi Trait Genotype Ideotype Distance Index (MGIDI) which is based on factorial analysis. This index assigns weightage to the individual traits according to the breeding goal and aids in selection of superior genotypes. The MGIDI index has been used by multiple researchers for selection of superior genotypes in barley (Pour-Aboughadareh *et al*., 2021), oats (Klein *et al*., 2023), sesame (Ahsan *et al*., 2024) and maize (Singamsetti *et al*., 2023).

The current study aims to estimate the genetic variability present in the maize germplasm, mine the trait associations through correlation analysis and selection of superior genotypes by defining ideotypes with differential maturity *i.e.* Late and medium maturity with high yield and employ the MGIDI index to select superior genotypes which are close to the defined ideotype.

2. Material and Methods

2.1 Experimental material and layout

A total of 55 germplasm lines were obtained from the maize unit, Department of Millets, Tamil Nadu Agricultural University, Coimbatore to carry out the research study. The experiment was performed in a Randomised Block Design with three replications during Rabi 2022-23 in the experimental farm of the Department of Millets. Throughout the crop season, standard agronomic procedures and plant protection practices were implemented. To carry out the current investigation, the biometrical observation on Days to 50% tasseling, Days to 50% silking, Plant height (cm), Cob length (cm), Cob diameter (cm), Number of kernel rows, Number of kernels/row, Shelling percentage (%), and Grain yield (g) were recorded in selected five plants per genotype.

2.2 Statistical Analysis

For each of the traits studied, an analysis of variance was performed. Burton and De Vane's (Burton and Devane, 1953) formula was used to calculate the coefficient of variation for these qualities. This variance was then categorised as high (more than 20%), moderate (10% - 20%), or low (less than 10%). The broad sense heritability and the projected genetic advance (GA) was calculated using (Johnson *et al.*, 1955) method and categorized as low, moderate, or high. Correlation analysis was carried out according to the methods described by (Miller *et al*., 1958). Principal Component Analysis, a dimension reduction approach developed by (Massey, 1965; Jolliffe, 1986) was used to compress variables while keeping important information. PCA was critical in decreasing data dimensionality and gaining insights from the dataset. The Multi-trait Ideotype Genotype Distance Index (MGIDI) distance index, established by (Olivoto and Nardino, 2020), was used to find genotypes that were closely related to the suggested ideotype. This ideotype is especially designed for early and late maturity periods, with the goal of producing a high yield. As a result, three ideotype reference models representing early, middle, and late maturity were developed. These reference models were used to identify genotypes that closely matched the target ideotype's attributes. To begin, each characteristic (rXij) was rescaled using the following equation (Eqn. 1)

$$rX_{ij} = \frac{\eta_{nj} - \varphi_{nj}}{\eta_{oj} - \varphi_{oj}} \times \left(\theta_{ij} - \eta_{oj}\right) + \eta_{nj} \quad \text{(Eqn. 1)}$$

where φ_{oj} and η_{oj} are the original minimum and maximum values for the trait *j*, respectively; φ_{nj} and η_{nj} are the new minimum and maximum values for trait *j* after rescaling, respectively; and θ_{ij} is the original value for *j*th trait of the *i*th genotype. The values for φ_{ij} and η_{ij} are chosen as follows: for the traits in which positive gains are desired, $\varphi_{nj} = 0$ and $\eta_{nj} = 100$ should be used, while for the traits in which negative gains are desired, $\varphi_{nj} = 100$ and $\eta_{ij} = 0$ should be used (Olivoto and Nardino, 2020). Subsequently, a factor analysis (FA) was carried out to facilitate the reduction of data dimensionality and to explore the underlying relationship structure. This analysis adhered to the following model (Eqn. 2)

$$F = Z\,(A^T\,R^{-1})^T \quad \text{(Eqn. 2)}$$

Where, F is a g × f matrix with the factorial score; Z is a g × p matrix with the rescaled means; A is a p × f matrix of canonical loading, and R is a p × p correlation matrix between the traits. Furthermore, g, f, and p indicates the number of genotypes, factor retained, and measured traits, respectively. In the third step, a [1 × p] vector was considered as the ideotype matrix. The MGIDI index was then calculated by calculating the Euclidean distance between the genotype scores and the ideal genotype values. The following equation (Eqn. 3) was used to achieve this calculation:

$$MGIDI_i = \sqrt{\sum_{j=1}^{f} (F_{ij} - F_j)^2} \qquad \text{(Eqn. 3)}$$

Where, $MGIDI_i$ is the multi-trait genotype-ideotype distance index for the *i*th genotype; Fij is the score of the *i*th genotype in the *j*th factor ($i = 1, 2, ..., g; j = 1, 2, ..., f$), being *g* and *f* the number of genotypes and factors, respectively, and Fj is the *j*th score of the ideotype. The genotype with the lowest MGIDI is then closer to the ideotype and therefore should presents desired values for all the analyzed traits.

GRAPES, an R-based programme created by (Gopinath *et al*., 2020), was used for the ANOVA and genetic variability parameters analysis. The "metan" package inside the R programme, (Olivoto and Lúcio, 2020), was used for Correlation analysis and MGIDI calculations. Furthermore, Principal Component Analysis (PCA) was performed with a mix of tools and packages. GRAPES was used in conjunction with the programmes ggplot2 (Wickham, 2016), corrplot (Wei and Simko, 2021), and factoextra (Kassambara and Mundt, 2020). These packages, used together, aided in the implementation of the PCA.

3. Results

3.1 *Per se* performance of the genotypes

Analysis of variance showed the presence of significant difference between all the traits indicating the presence of ample genetic variation for further exploitation (Supplementary Table 1). The *per se* performance (Table 2) for the observed traits in the experiment revealed the following trends. Plant height ranged between 108 to 182 cm with a mean of 142.46 cm. while days to 50% tasseling ranged between 50 to 65 days categorizing the germplasm into medium and late duration types, while the days to 50% silking ranged between 52 to 68 days. The cob length ranged between 10.0 to 18.0 cm with an average length of 14.7 cm, while the cob diameter ranged between 3.50 cm to 8.10 cm with an average diameter of 6.40 cm. kernel rows per cob ranged between 10 to 18 rows with a mean of 14 rows per cob, while the kernel number per row ranged between 12 to 37 kernels per row with an average

Table 1: List of 55 genotypes used under study

Code	Genotypes	Code	Genotypes
G1	B. No 9119-1-1	G29	B. No 9233-1
G2	UMI 1151-2	G30	UMI 1113
G3	UMI 653-2-3	G31	B. No 1125-7
G4	Hyd No 1075-4-1-1	G32	B. No 1258-7
G5	UMI 819-3	G33	UMI 96
G6	B. No 1265-6-2	G34	UMI 1051
G7	UMI 1101	G35	Hyd No. 2009-2-2-15
G8	B. No 1076-5-1	G36	B. No 1110-8
G9	B. No 71810	G37	B. No 1076-5-4-1
G10	UMI 1003-2-3	G38	Hyd No. 1082-2
G11	UMI 178	G39	UMI 142
G12	B. No 72183-9-2	G40	B. No 426-3
G13	B. No 1043-7	G41	B. No 71806
G14	B. No 1131-5	G42	Hyd No. 1075-4-2
G15	B. No 1421-5-1	G43	B. No 1076-5-4-3
G16	UMI 164	G44	UMI 346-1
G17	B. No 1118-3	G45	9119-1-2-1
G18	UMI 406	G46	UMI 504
G19	UMI 697-2	G47	B. No 1253-8
G20	UMI 1131-1	G48	UMI 920
G21	B. No 1075-2	G49	UMI 1098-4
G22	B. No 1917-2-1-1	G50	UMI 823
G23	UMI 1105	G51	UMI 1223
G24	B. No 1064-5	G52	UMI 1210
G25	B. No 1266-7	G53	UMI 1205
G26	UMI 1009-2-2	G54	UMI 1220
G27	B. No 1048-7	G55	UMI 1201
G28	B. No 1076-5-4-2		

of 28 kernels per row. The shelling percent ranged from 75.0 to 82.5% with an average of 79%. The grain yield ranged between 42.4 g to 77.5 g with a mean yield of 61.20 g.

3.2 Genetic variability

The components of genetic variability such as phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), broad-sense heritability are given in Table 2 and discussed below.

3.3 Components of genetic variance

The Phenotypic coefficient of variance is higher than the genotypic coefficient of variance indicating the presence of environmental effect in

Table 2: Best performing maize inbred lines for yield and yield attributing traits

Sl. No	Genotype	Grain yield (g)	PH	DFT	DFS	CL	CD	NKR	NKP	SP
1	UMI 1009-2-2	77.5	143.7	57	60	17.2	7.0	18	33	79.0
2	UMI 1131-1	75.3	182.0	55	57	17.0	7.9	18	32	78.6
3	B. No 1048-7	75.0	162.2	56	59	18.0	7.1	16	37	78.9
4	B. No 1076-5-1	74.7	134.8	51	54	17.7	7.0	16	35	79.8
5	UMI 96	74.5	168.7	54	57	17.4	6.6	16	37	82.0
6	B. No 1110-8	74.5	108.0	53	55	16.5	7.0	16	32	80.2
7	9119-1-2-1	74.3	126.1	57	59	17.6	6.5	18	32	80.7
8	UMI 1201	74.1	162.4	53	56	17.0	6.2	16	37	79.4
9	Hyd No. 2009-2-2-15	73.6	118.8	58	62	17.0	6.8	16	36	79.0
10	UMI1210	73.0	158.0	52	55	16.0	7.0	16	35	79.2
Minimum		42.4	108	50	52	10.1	3.45	10	12	75
Maximum		77.5	182	65	68	18	8.05	18	37	82.5
Mean		61.20	142.46	55	58	14.75	6.40	14	28	79.00
PCV (%)		17.59	13.5	5.54	5.36	15.69	16.88	18.15	22.9	3.64
GCV (%)		16.17	13.12	5.24	4.95	14.88	12.37	12.08	20.82	1.19
h^2_{bs} (%)		84.5	94.4	89.6	85.2	89.9	53.7	44.3	82.6	10.7
GAM (%)		30.6	26.3	10.2	9.4	29.1	18.7	16.6	39	0.8

PH = Plant height (cm), DFT = Days to 50% flowering, DFS = Days to 50% silking, CL = Cob length (cm), CD = Cob diameter (cm), NKR = Number of kernel rows, NK = Number of kernels per row, SP = Shelling percentage (%), GY = Grain yield (g)

the trait expression. Further, High values for both PCV and GCV were exhibited by kernel number per row. Kernel row number, grain yield, cob diameter, cob length and plant height exhibited moderate values for both PCV and GCV, while days to 50% tasseling, days to 50% silking and shelling percentage showed low PCV and GCV values. The magnitude of difference between the PCV and GCV estimates can be used to determine the amount of variance that occurs due to environment.

3.4 Broad sense heritability (h^2_{bs}) and Genetic Advance as percent of Mean (GAM)

Plant height, cob length, kernels per row and yield exhibited high heritability coupled with high genetic advance. Days to 50% tasseling, days to 50% silking exhibited high heritability with moderate GAM. Cob diameter and kernel row number exhibited moderate values for both heritability and genetic advance. While, shelling percentage exhibited low heritability and genetic advance.

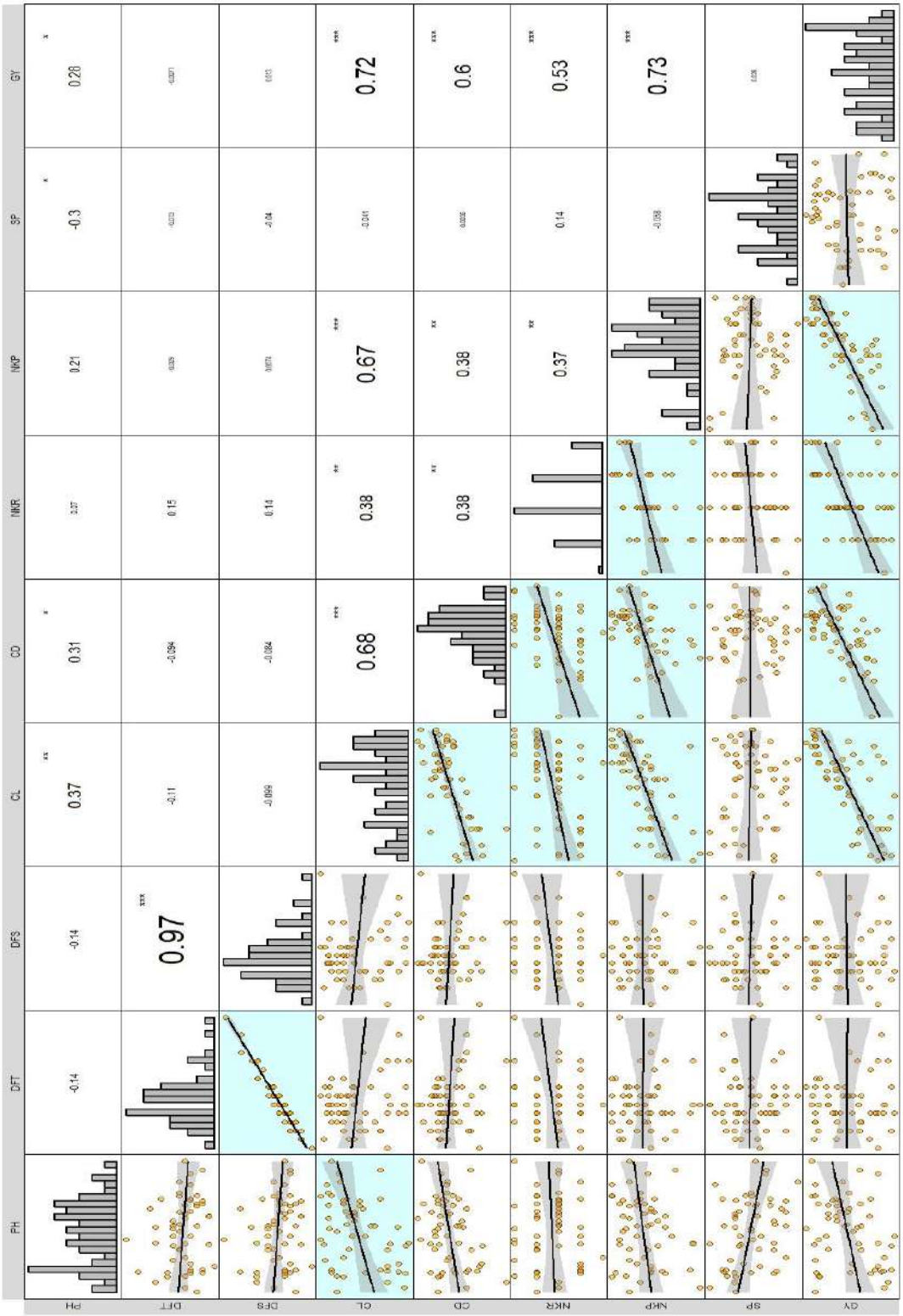
3.5 Correlation

Correlation analysis for the traits studied revealed various significant trait associations (Fig. 1). Days to 50% tasseling and days to 50% silking showed high positive significant correlation between each other. Yield is a trait which is influenced by multiple dependent factors, hence an analysis of the trait association with the grain yield reveals the traits to be improved to achieve higher yields. Grain yield significant positive association with plant height, cob length, cob diameter, number of kernel rows, number of kernels per row. Hence selection for these traits can be utilized to improve grain yield.

3.6 Principal component analysis

The principal component analysis method, established by Karl Pearson in 1901, is used to reduce the size of a data set into a number of components (Venujayakanth *et al.*, 2017). The Principal Component Analysis (PCA) conducted in this study effectively partitioned the total variability into nine principal components, where three components displayed eigenvalues surpassing one, signifying their substantial contribution in explaining the dataset's variance as evidenced in the scree plot (Fig. 2). Collectively, these three principal components accounted for a noteworthy 74.44% of the overall variance. Notably, the first PC emerged as the most influential, explaining 37.37% of the total variance, followed by the second (22.89%) and the third (14.17%) PCs. The identified trait contributions delineated distinct patterns: the first PC was characterized by traits associated with grain yield, cob characteristics, except number of kernel rows, while the second PC predominantly encapsulated flowering traits. The third PC prominently featured shelling percentage and plant height, elucidating their influence on this component. The trait number of kernel rows was the major contributor for the variance explained by PC6 (Fig. 2).

PCA biplot exhibits the relation between the traits and between the genotypes (Fig. 3). The acute angle between two traits reveals the positive association between them, while an angle >90° indicated negative association. The flowering traits are in high positive correlation with each other, while the yield and cob traits such as cob length, cob diameter, kernel row number, kernels per row showed positive associations amongst them. Based on their scatter position on the biplot,



PH = Plant height (cm), DFT = Days to 50% flowering, DFS = Days to 50% silking, CL = Cob length (cm), CD = Cob diameter (cm), NKR = Number of kernel rows, NK = Number of kernels per row, SP = Shelling percentage (%), GY = Grain yield (g)

Fig. 1 Correlation analysis for all the nine traits under study

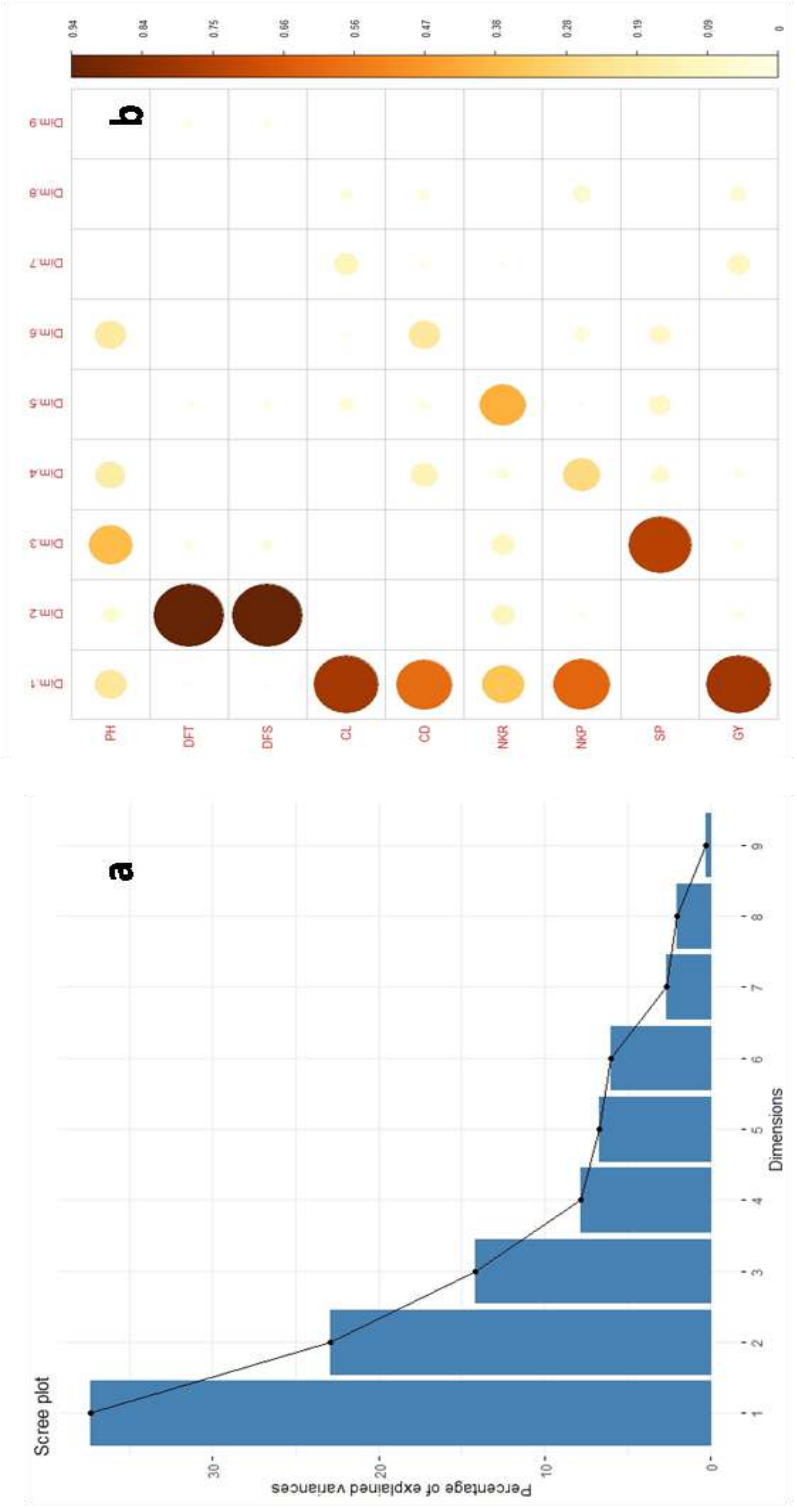
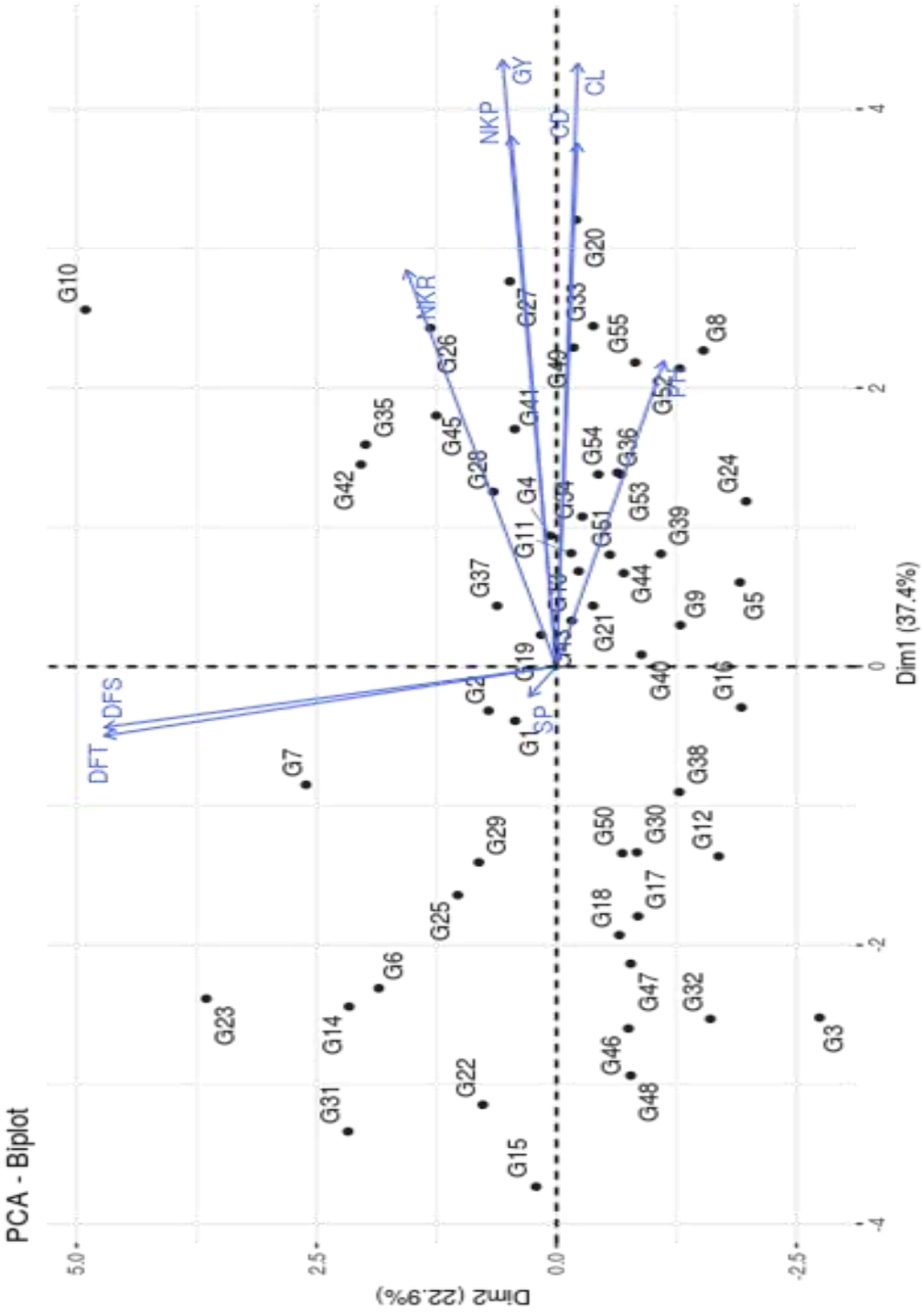


Fig. 2 (a) Scree Plot for all principal components and (b) contribution of different traits to the variance explained by each principal component



PH = Plant height (cm), DFT = Days to 50% flowering, DFS = Days to 50% silking, CL = Cob length (cm), CD = Cob diameter (cm), NKR = Number of kernel rows, NK = Number of kernels per row, SP = Shelling percentage (%), GY = Grain yield (g)

Fig. 3 PCA Biplot for both traits and Genotypes

the genotypes UMI 1003-2-3 (G10), B. NO 1421-5-1 (G15), B. NO 1917-2-1-1 (G22), G3, B. NO 1125-7 (G31), UMI 920 (G48) are placed the farthest in the biplot indicating the diverse nature of these genotypes.

3.7 Multi trait Genotype Ideotype Index

Initially two ideotypes were defined with varying maturity *i.e*. medium and late, maturing with high yield. The MGIDI distances to selected genotype were calculated with respect to each ideotype. A selection intensity of 15% is opted for the selection criteria (Fig. 4).

3.8 Medium maturing ideotype

The selection gains (Table 3) were positive for all the traits with kernels per row having the highest gain at 18.60% followed by grain yield 18.30%, cob length (14.80%). Shelling percentage has the least gains at 0.07%. Cumulative selection gain was recorded at 70.21%. Eight genotypes were selected at 15% SI, which are B. NO 1265-6-2 (G8), UMI 1210 (G52), UMI 96 (G33), UMI 1201 (G55), UMI 1098-4 (G49), B. No 1110-8 (G36), B. NO 1064-5 (G24), UMI 1205 (G53). The genotype B. NO 1265-6-2 (G8) was the closest with least MGIDI value of 1.10 followed by UMI 1210 (G52) (1.59), UMI 96 (G33) (1.69) (Table 3).

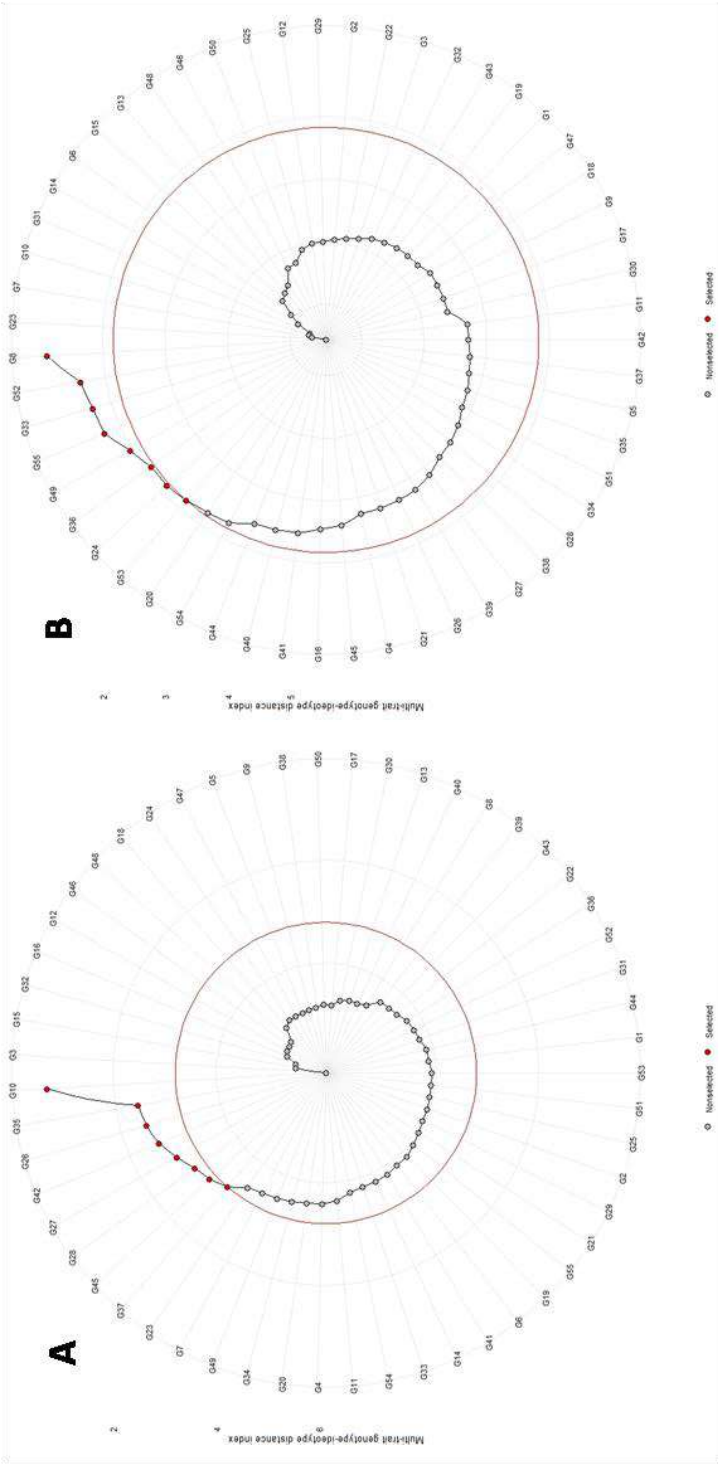
3.9 Late maturing ideotype

All the traits showed positive selection gains (Table 3) ranging from 18.80% for Kernel number to 0.03% for shelling percentage, except for plant height which showed a negative selection gain (-5.31). A cumulative selection gain was recorded at 69.06% and a negative selection gain of -5.31%. Similarly, eight genotypes were selected based on SI of 15% UMI 1003-2-3 (G10) HYD NO. 2009-2-2-15 (G35), UMI 1009-2-2 (G26), Hyd No. 1075-4-2 (G42), B. NO 1048-7 (G27), B. NO 1076-5-4-2 (G28), 9119-1-2-1 (G45), B.NO1076-5-4-1 (G37) with UMI 1003-2-3 (G10) having the closest resemblance to the proposed ideotype based on its MGIDI values (0.71) (Table 3).

Table 3: Factor analysis and Multi Trait Genotype Ideotype Distance Index for two ideotypes

Trait	Factor	Eigen Value	PVE	CPVE	SG_{perc}		Genotypes selected			
					Medium	Late	Medium	MGIDI	Late	MGIDI
CL	FA1	3.36	37.37	37.37	14.80	12.40	G8	1.10	G10	0.71
CD					5.98	5.78	G52	1.59	G35	2.44
KRN					7.40	5.99	G33	1.69	G26	2.50
NKP					18.60	18.80	G55	1.73	G42	2.61
GY					18.30	17.00	G49	1.98	G27	2.81
DFT	FA2	2.06	22.89	60.26	1.47	4.47	G36	2.12	G28	3.00
DFS					1.23	4.59	G24	2.12	G45	3.07
PH	FA3	1.28	14.17	74.44	2.36	-5.31	G53	2.17	G37	3.21
SP					0.07	0.03				
Cumulative selection gain					70.21	69.06				
Negative selection gain						-5.31				

PH = Plant height (cm), DFT = Days to 50% flowering, DFS = Days to 50% silking, CL = Cob length (cm), CD = Cob diameter (cm), NKR = Number of kernel rows, NK = Number of kernels per row, SP = Shelling percentage (%), GY = Grain yield (g); PVE = Percent variance explained; CPVE= Cumulative percent variance explained



A = MGIDI view for Late maturing ideotype, B = MGIDI view for medium maturing ideotype

Fig 4. Multi Trait Genotype Ideotype Distance Index for late and medium maturing ideotypes

3.10 Strength and Weakness view of selected genotypes

The radar plot depicts the strength and weaknesses of the selected genotypes for various maturity periods are visualized (Fig. 5). Smaller proportions explained by a factor that is placed closer to the external edge indicate that the trait within that factor is more similar to the ideotype (Singamsetti *et al.*, 2023). A view on strength and weakness under medium maturity showed that the genotypes B. NO 71806 (G41) and G56 showed strengths related to factor 1 which holds yield and yield attributing traits, whereas B. NO 1064-5 (G24) and B. NO 1265-6-2 (G8) showed strengths for the factor 2 which holds flowering traits (DFT, DFS), and UMI 96 (G33), UMI 1098-4 (G49), B. NO 1265-6-2 (G8) showed strengths in factor 3 which holds SP and PH.

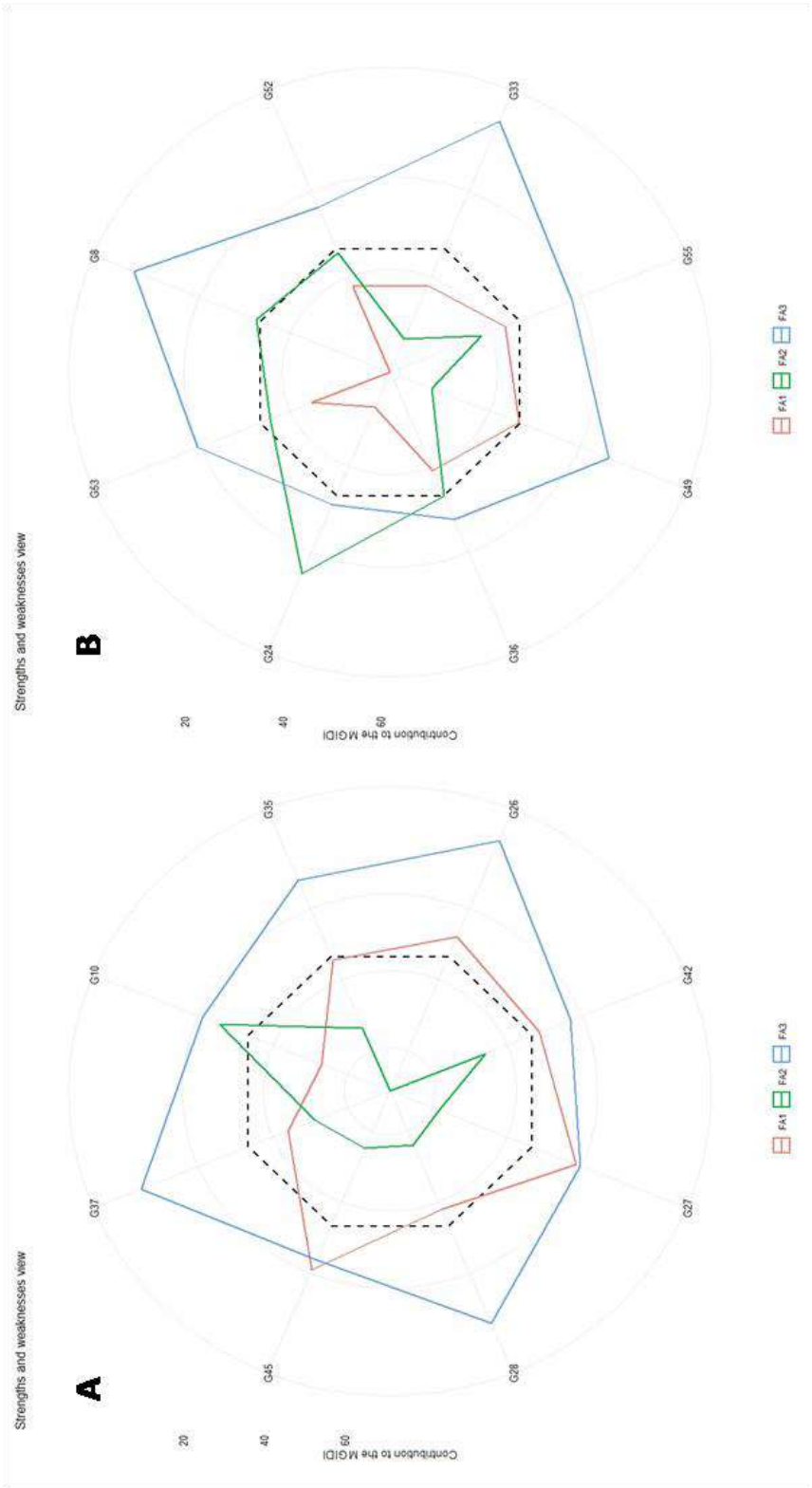
Similarly, for late maturity, the genotypes 9119-1-2-1 (G45) and B. NO 1048-7 (G27) showed strength in FA1, while UMI 1003-2-3 (G10) showed strength in FA2 and B. NO 1076-5-4-1 (G37), B. NO 1076-5-4-2 (G28), and UMI 1009-2-2 (G26) showed strength in FA3.

4. Discussion

A successful maize breeding program relies on the amount of genetic variability present in the available germplasm. Wider presence of genetic variability aids in selection of desirable genotypes. The estimates of PCV and GCV explain the amount of variation present as expressed by either the genotype or the environment. In this study, high values for both PCV and GCV were exhibited by kernel number per row. Kernel row number, grain yield, cob diameter, cob length and plant height exhibited moderate values for both PCV and GCV. The magnitude of difference between the PCV and GCV estimates can be used to determine the amount of variance that occurs due to environment. Similar results were reported by other researchers (Netaji *et al.*, 2000; Nagabhushan *et al.*, 2011; Kapoor and Batra, 2015; Kandel *et al.*, 2018; Prakash *et al.*, 2019).

Estimates of genetic variability coupled with heritability and genetic advance estimates aid us in selection and improvement programs to obtain desirable genetic gains (Swarup and Chaugle, 1962). The values of heritability and genetic advance as mean, together can be used to determine the mode of gene action that is underlying the expression of the particular trait. A high heritability and GAM value indicate additive gene action, while a high heritability and low GAM percent indicate non-additive gene action. Low heritability and high GAM values indicated additive gene action but the heritability value could be due to environmental influence. Low heritability and GAM values show non additive gene action and also the prevalence of environmental influence in the manifestation of the character under study. In this study, traits like plant height, cob length, kernels per row and yield are influenced by additive gene action, so selection for improvement of these traits can be followed for achieving desired gains. Shelling percentage based on its heritability and GAM values, it can be deduced that the underlying gene action controlling its expression is of non-additive type. (Nagabhushan, 2011; Sofi *et al.*, 2007; Panwar *et al.*, 2013; Rahman *et al.*, 2017) reported similar results for these traits.

Traits that contribute to yield are termed as yield attributing traits and these are generally in positive association with the yield. Positive, significant and high associations can be observed between cob length, cob diameter, number of kernel rows, and



A = Strength and weakness view of genotypes selected for late maturing ideotype, B = Strength and weakness view of genotypes selected for medium maturing ideotype

Fig 5. Strength and Weakness view of genotypes selected for late and medium maturing ideotype

number of kernels per row with yield (Rafiq *et al.*, 2010; Munawar *et al*., 2013; Lakshmi *et al.*, 2018; Rai *et al*., 2021). Hence, selection for improvement of these traits can be used to achieve yield improvement.

The study utilized Principal Component Analysis (PCA) to decompose total variability into nine components, where three components, accounting for 74.44% of variance, exhibited eigenvalues >1. Trait contributions highlighted distinct patterns: PC1 correlated with grain yield, cob traits; PC2 predominantly linked to flowering traits, while PC3 emphasized shelling percentage and plant height while the variation captured by PC6 was explained by number of kernel rows. The similar work has been done by (Okporie, 2008; Iqbal *et al.*, 2015; Bhusal *et al*., 2016). Varimax rotation reinforced the 'number of kernel rows' association with PC1. High communality values (98% to 53%) indicated substantial collective variance explanation for traits. The placement of the genotypes far from the origin revealed their diversity compared to other genotypes. The genotypes UMI 1003-2-3 (G10), B. NO 1421-5-1 (G15), B. NO 1917-2-1-1 (G22), G3, B. NO 1125-7 (G31), UMI 920 (G48) were placed in the either extremes of the biplot, hence explaining their diverse nature. The genotypes can be employed for their utilization in breeding programs.

The identification of medium and late maturing genotypes is crucial for producing hybrids preferred in specific Indian regions, emphasizing the importance of selecting genotypes with targeted traits in breeding programs. The MGIDI index offers a clear and effective selection method with practical applications for long-term genetic improvement. Assessing strengths and weaknesses using this approach provides a valuable tool to pinpoint areas for trait enhancement, distinguishing it from other indices. For instance, genotype UMI 1003-2-3 (G10) excels in flowering traits aligning with the proposed late maturing ideotype, while B. NO 1265-6-2 (G8), despite underperforming in flowering traits, suggests potential for improving yield traits while maintaining medium maturity. This approach was utilized by (Klein *et al*., 2023; Shirzad *et al*., 2022; Palaniyappan *et al*., 2023; Lima *et al*., 2023) in different crops to select genotypes that closely resemble the ideotypes suited for their breeding objective.

5. Conclusion

The presence of genetic variability is a requisite for any successful breeding program. Successful exploitation of the available genetic variability depends on the efficiency of selection based on the breeding objectives. Genotypes UMI 1009-2-2, UMI 1131-1, B. No 1048-7, B. No 1076-5-1, UMI 96, B. No 1110-8, 9119-1-2-1, Hyd No. 2009-2-2-15, UMI 1210 showed higher yield amongst other genotypes. Assessment of the association of traits provides an understanding, on trait interrelationships which can aid in further improvement. Every breeding objective has an ideotype around which the objective is focused on. Selection for single traits may increase the gains of that particular trait, but in process there is a chance of overlooking complex interactions between the studied traits. MGIDI is a comprehensive approach which enables simultaneous selection of genotypes based on multiple traits. Cumulative positive selections gains obtained for medium maturing ideotype was 70.21%, while it was 69.06% for late maturing ideotype with grain yield and number of kernels per row contributing the highest selection gains. Negative selection gains were observed in plant

height for late maturing ideotype. At a selection intensity of 15%, 8 genotypes were selected which lie closer to the ideotype. Hence, utilization of a multi trait based selection index can enable effective selection of genotypes without any loss of variability.

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The impact of resource efficiency on castor production in the Banaskantha district of Gujarat

Pragneshkumar K Patel[1] and **Bhaveshkumar Parmar**[2]
[1]Academic Coordinator, Ascent School of Science, Palanpur, Gujarat, India.
[2]Assistant Professor, National Forensic Sciences University, Gujarat, India

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ABSTRACT

The Government of India considers the cost of agriculture as an important budgetary indicator. Therefore, this study was conducted to examine the rates of development in the area, production, and productivity, as well as the efficiency of resource utilisation in the cultivation of castor crop in the Banaskantha district of Gujarat state. The researchers utilised a multistage sampling strategy to choose the farms. An analysis was conducted on the talukas with the largest area dedicated to cultivating castor crops. One hundred farmers were chosen at random for this experiment. The data was analysed using analytical tools such as the Cobb Douglas production function and exponential growth function.

The study revealed that the compound growth rates (CGRs) for the area, production, and productivity of castor crops in the Banaskantha district have shown positive and highly significant trends over the past twenty years, with growth rates of 3.53%, 5.86%, and 2.24% respectively. However, the area under castor cultivation in both the Banaskantha district and the entire state of Gujarat has experienced negative growth rates of -4.09% and -0.9585% respectively over the past ten years. The MVP/FC ratio for the cost of human work, bullock and tractor charges, cost of manure, and cost of fertiliser and plant protection were calculated to be 3.66, 4.19, 2.84, 2.62, and 1.27 correspondingly. These values indicate that these resources were utilised inefficiently, with an underuse of resources. The negative cost-to-seed ratio suggests that there was an over usage of seeds. Additionally, the irrigation expenses of 0.82 indicate an overuse of this resource. The total of regression coefficients (Σbi's) was 1.035, which suggests that there is a rising return to scale (stage-I) on castor farms. Therefore, it is necessary to enhance the allocation of resources that were not fully utilised and decrease the allocation of resources that were excessively utilised in order to maximise the yield from castor production.

KEY WORDS: *Castor*; *Resource use efficiency*; *Cobb-Douglas production function*; *Cost of cultivation*; *Compound growth rate.*

1. Introduction

The agriculture scenario of the country in general and Gujarat State, in particular, is changing over the years. Gujarat is one of the leading producers of agricultural crops in the country and known for its varied agro-climatic conditions and cropping pattern (Aqaayar *et al.*, 2019). There is huge scope to increase the area and production of castor crop in the Banaskantha as well as in the state. But this crop has some associated problems which hamper growth. The information on resource use efficiency and input utilization pattern will be useful to the crop growers in a reallocation of their resources to improve the production of castor crop. The results of the study give information on

CONTACT Pragneshkumar K Patel pkp_bba@yahoo.com

growth and resource use efficiency which would be useful to the producers, researchers, and policymakers to take appropriate measures to improve productivity of castor (Kumar *et al*., 2016; Sujan *et al*., 2017). Hence, the present study was undertaken to analyse growth and resource use efficiency of castor cultivation in Banaskantha district of Gujarat state with the aiming to assess Banaskantha district castor crop growth, production, and productivity and castor crop resource usage efficiency.

2. Material and Methods

The current investigation was conducted in the Banaskantha district located in North Gujarat. In order to assess the goals of the study, data were gathered from primary and secondary sources. The researchers employed a multistage sampling technique to choose the farmers for the study. The Banaskantha district was deliberately chosen for the study due to its favourable agro-climatic conditions and soil types for growing castor crops. For the second stage, Palanpur taluka was deliberately chosen due to its largest cultivated area of castor crop compared to other talukas in the Banaskantha district. During the third step, a random selection was made of five villages from the pool of villages where castor is grown in the taluka. During the ultimate phase, 20 farmers were randomly chosen from those who have cultivated the castor crop, to be selected as representatives from each of the chosen communities. Hence, a total of 100 farmers engaged in castor cultivation were selected at random from the Banaskantha area for the purpose of this study.

2.1 Data Analysis

The exponential function was used for measurement of trend and the Compound Annual Growth Rate (CGR) of yield, area and total production of castor cultivation. The production and income of farmers from cultivation of castor crop were influenced by various factors. Therefore, the production function approach will be used to find out the efficiency of resources used in the cultivation of castor crop. For this purpose, the Cobb-Douglas production function was employed. For achieving the specific objective of the study, the Cobb-Douglas form of production function was estimated taking gross income as dependent variable and cost of human labour, bullock and tractor charges, cost of manures, cost of fertilizer, the value of seeds, cost of irrigation and cost of plant protection as independent variables of selected crops. The Cobb-Douglas production function was employed as below:

$$Y = a \cdot X_1^{b1} \cdot X_2^{b2} \cdot X_3^{b3} \cdot X_4^{b4} \cdot X_5^{b5} \cdot X_6^{b6} \cdot X_7^{b7} \cdot U \ldots\ldots\ldots (1)$$

Where, Y = Gross income (₹)

a = Intercept

X_1 = Cost of human labour (₹)

X_2 = Bullock and tractor charges (₹)

X_3 = Cost of manures (₹)

X_4= Cost of fertilizer (₹)

X_5 = Cost of seeds (₹)

X_6 = Cost of irrigation (₹)

X_7 = Cost of plant protection (₹)

bi = Production elasticity of respective inputs (X_i's), n

Σ bi = Returns to scale, and i=1

U_t = Error term with usual assumptions

3. Results and Discussion

The results of the various aspects are presented under the following heads.

3.1 Growth in Area, Production, and Productivity of castor crop

For the computation of Compound Annual Growth Rate (CAGR) of area, production and productivity of castor in Banaskantha district, as well as Gujarat state data, were collected from the year 2000-01 to 2019-20. It is divided into three Periods, Period - I (2000-01 to 2009-10), Period-II (20010-11 to 2019-20), and overall Period (2000-01 to 2019-20).

It is evident from the Table 1 the compound growth rates (CAGR) of castor crop in Banaskantha district were found positive and highly significant *i.e.*, 3.53 % for area, 5.86 % for production and 2.24 % per annum for yield during last twenty years (2000-01 to 2019-20). In case of Gujarat state, there was positive and highly significant compound growth rates 7.28 %, 2.16 %, 5.01 % were recorded for yield of castor, production and area of castor during this period (2000-01 to 2019-20) respectively. It indicates that the trend of production of castor in Banaskantha district and the Gujarat state was expended due to rise in both area and productivity.

The period wise growth rate revealed that from the year 2010-11 to 2019-20 (period-II), the compound growth rates of castor in Banaskantha district were estimated -4.09 % for area and -2.32 % per annum for production and at Gujarat state level, it was also found negative -0.95 % and -0.33 % per annum, respectively. This is mainly due to the diversification of crops. Considering the period wise growth, the area and production growth showed a decline trend in period-II as compared to period-I. But during the period-II, the yield growth was raised, this might be due to the adoption of new high yielding seed varieties and new technology in castor cultivation, the farmers gained higher production per unit despite slight change in area. This decline change in area was affected in change in crop growing pattern of castor grower in study area.

It may be concluded that area under castor, production and yield of castor in Banaskantha and the Gujarat state increased significantly in the overall period (2000-01 to 2019-20). The period wise growth rate analysis revealed that during the period-II (2010-11 to 2019-20), the growth rates of area of castor and production showed negative growth while productivity achieved positive growth in castor in Banaskantha district.

Table 1: Compound growth rate (CGR) of castor in Banaskantha district and Gujarat state

Sl. No.	Periods	Area (in %)	Production (in %)	Yield (in %)
A	Banaskantha district			
1	Period - I (2000-01 to 2009-10)	3.7467*	6.1994*	2.4028
2	Period - II (2010-11 to 2019-20)	-4.0995	-2.3248	1.8558
3	Overall Period (2000-01 to 2019-20)	3.5367**	5.8613**	2.2450**
B	Gujarat State			
1	Period - I (2000-01 to 2009-10)	2.4934	7.2889*	4.6792**
2	Period - II (2010-11 to 2019-20)	-0.9585	-0.3296	0.6319
3	Overall Period (2000-01 to 2019-20)	5.0125**	7.2827**	2.1616**

* Significance level 0.05 ** Significance level 0.01

3.2 Resource Use Efficiency of Castor Cultivation

The regression co-efficient of inputs used for castor production were obtained from the Cobb Douglas production function for sample farmers are presented in Table 2.

It is seen from Table 2 that the value of regression co-efficient of production was found negative as well as positive. The value of co-efficient of production of castor was ranged from -0.025 (cost of seeds) to 0.490 (cost of human labour). It is inferred that among the explanatory variables of castor production, the value of co-efficient of cost of human labour (X_1 = 0.490), bullock and tractor charges (X_2 = 0.306) and cost of manure (X_3 = 0.048) were positive and highly significant while in case of cost of fertilizers (X_4 = 0.094) and cost of irrigation (X_6 = 0.104) were positive and significant showing positive impact on gross income of castor. The cost of seeds explained negative (X_5 = -0.025) and non-significant indicated negative impact on gross income of castor, whereas the cost of plant protection (X_7 = 0.018) explained positive, but non-significant showing positive impact on gross income of castor.

The co-efficient of multiple determinations (R^2) was 0.944 indicating that 94 % of the total variation in the gross income from castor was explained by the explanatory variables included in the function. The sum of regression co-efficient (Σbi's - return to scale) was more than one (1.035) in castor cultivation indicating increasing return to scale. Thus, it can be concluded that gross return from castor crop proportionately increased at increasing rate with raise in the variable factors.

3.3 Efficiency Levels of Inputs Utilised In Castor Cultivation

For evaluating the efficiency of resource use, the ratio of the marginal value products (MVP) to marginal factor cost (MFC) for all variables were calculated and the findings are given in Table 3.

The MVP/MFC ratio of bullock and tractor charges, cost of human labour, cost of manure and cost of fertilizer and plant protection were estimated 4.19, 3.66, 2.48, 2.62 and 1.27 respectively. These values were positive and more than one indicating those resources were used inefficiently *i.e.*, underutilized.

Table 2: Estimated Cobb Douglas production function for castor

Sl. No.	Explanatory Variables	Regression Coefficients (bi)	Standard Error	t- stat value	Sig. P-value
1	X_1 = Cost of human labour (₹)	0.490 **	0.0590	8.304	0.0001
2	X_2 = Bullock and tractor charges (₹)	0.306 **	0.0574	5.326	0.0001
3	X_3 = Cost of manures (₹)	0.048 **	0.0172	2.819	0.0058
4	X_4 = Cost of fertilizers (₹)	0.094 *	0.0448	2.108	0.0377
5	X_5 = Cost of seeds (₹)	- 0.025	0.0466	-0.543	0.5881
6	X_6 = Cost of irrigation (₹)	0.104 *	0.0478	2.169	0.0326
7	X_7 = Cost of plant protection (₹)	0.018	0.0271	0.655	0.5136
	a = Intercept	0.908	0.1179	7.698	0.0001
	Σbi's = Returns to scale	1.035	-	-	-
	R^2 = Coefficient of determination	0.944	0.0599	-	-
	Calculated F-value and P-value	255.09 **	-	-	0.0001

* Level of significance 0.05 ** Level of significance 0.01

The production function analysis of castor gave negative value of MVP/MFC ratio of cost of seeds (-2.07) indicated excessive use of this resource, while MVP/ MFC ratio was less than one in case of irrigation (0.82) showed over use of this resource in castor cultivation. Therefore, castor farmers can raise gross returns by reducing cost of seeds and cost of irrigation.

It can be concluded that the above resources use analysis in castor farms indicated that there is considerable scope for raising the efficiency of resources use in castor by readjustment of resources. Hence, the resources which were underutilized should be increased and those which were over utilized should be reduced in order to optimize the return from castor production.

4. Conclusion

- The compound growth rates for the area, production, and productivity of castor crops in the district were positive and highly significant *i.e.*, 3.53, 5.86, and 2.24 % respectively during the last twenty years whereas the area under castor in Banaskantha district and Gujarat state level, however negative compound growth rate (-4.09 % and -0.9585 %) was found over last ten years, respectively. Therefore, considering the agro climatic condition of the district, efforts should be made by the policy maker to increase the area and productivity of the castor crop (Sinha *et al*., 2019; Veeranagouda *et al*., 2011).

- Among the explanatory variables, the value of elasticity of cost of human labour and bullock and tractor charges, cost of manure, fertilizer and cost of irrigation were positive and significant showing positive impact on gross income of castor while the cost of seeds explained negative and non-significant indicated a negative impact on the gross income of castor (Salve *et al*., 2017).

- The MVP/FC ratio of cost of human labour, bullock and tractor charges, cost of manure and cost of fertilizer and plant protection were 3.66, 4.19, 2.84, 2.62 and 1.27 respectively, indicating these resources were used inefficiently (under use of resources). Therefore, the gross

Table 3: Marginal value product (MVP) to marginal factor cost (MFC) ratio for castor cultivation

Sl. No.	Explanatory Variables	Marginal value product (MVP)	Factor cast (MFC)	Ratio of MVP to MFC (r)	Resource use efficiency status
1	X_1 = Cost of human labour (₹)	3.66	1.00	3.66	Under
2	X_2 = Bullock and tractor charges (₹)	4.19	1.00	4.19	Under
3	X_3 = Cost of manures (₹)	2.84	1.00	2.84	Under
4	X_4 = Cost of fertilizers (₹)	2.62	1.00	2.62	Under
5	X_5 = Cost of seeds (₹)	- 2.07	1.00	-2.07	Over
6	X_6 = Cost of irrigation (₹)	0.82	1.00	0.82	Over
7	X_7 = Cost of plant protection (₹)	1.27	1.00	1.27	Under
	Σbi's = Returns to scale	1.035			

MVP/MFC ratio > 1 indicates under utilization of resource
MVP/MFC ratio < 1 indicates over utilization of resource
MVP/MFC ratio = 1 indicates efficiently utilization of resource

returns can be increased by using more of these five inputs in castor cultivation.

- The negative ratio of cost of seeds indicated excessive use while it was less than one in case of irrigation (0.82) showed over use of these resources in castor cultivation. Therefore, farmers can increase gross returns by reducing these two resources.

- The sum of regression co-efficient (Σbi's) was 1.035 (more than one) indicating increasing return to scale (stage-I) on castor farms. Hence the resources which were underutilized should be increased and those which were over utilized should be reduced in order to optimize (maximizing) the return from castor production. Therefore, there is an enough potentiality of raising castor production by readjustment of resources.

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Variability studies in F_3 populations of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) for yield and yield contributing traits

K Manasa, **E Padma, G Kranthi Rekha, M Paratpara Rao** and **K Umakrishna**
Department of Vegetable Science, College of Horticulture, Dr. YSR Horticulture University, Venkataramannagudem - 534101, West Godavari, Andhra Pradesh, India

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ABSTRACT

Genotypic coefficient of variation and phenotypic coefficient of variation, heritability and genetic advance were undertaken for sixteen characters of five crosses of bottle gourd in F_3 generation. High genotypic and phenotypic coefficient of variation and high heritability estimates associated with high values of genetic advance as a percent mean were observed for fruit yield per plant in all the five crosses, average fruit weight in Pusa Sandesh × Arka Bahar and Pusa Naveen × Pusa Santhusti, number of fruits per vine in Pusa Sandesh × Arka Bahar, Pusa Naveen × Pusa Santhusti and Pusa Naveen × Local Round, number of seeds per fruit in Pusa Naveen × Pusa Santhusti which indicated additive gene action for these characters, which could be improved by simple selection method.

KEY WORDS: *Bottle gourd*; *Genotypic coefficient of variation*; *Phenotypic coefficient of variation*; *Heritability*; *Genetic advance*

1. Introduction

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is an annual monoecious species belongs to the family Cucurbitaceae with Chromosome number 2n=22. Bottle gourd is one of the cultivated tropical and subtropical vine and it is commonly known as calabash gourd, white flowered gourd, lauki, ghia *etc*., The term *Lagenaria siceraria* is derived from two Latin words *lagena* which means bottle and *sicera* means drinking utensil. Bottle gourd is originated in Africa with a long history of cultivation in Asia and other warmer regions of the world. Secondary centre of origin of bottle gourd is India with a very good repository of diverse germplasm.

The main prerequisite for launching a breeding programme is the extent of genetic variability and genetic divergence in breeding material. Wide differences between morphological traits such as size, colour, resistant to pests and diseases and yield are of immense importance to the breeder since number of cultivars could be developed to suit various requirements. As the area and production of bottle gourd are increasing fast but the crop still remains less explored on aspects of crop improvement by breeding methods. Thus, there is much need of cultivars with early fruiting, high yield, and high female to male ratio, medium sized fruits. Therefore, to introgress these horticultural traits, the F_3 progenies were assessed

CONTACT K Manasa manasakosinepalli1997@gmail.com

for variability, heritability and genetic advance for the utilization in crop improvement.

2. Material and Methods

The experiment was conducted at College of Horticulture, Dr. Y. S. R. Horticultural University, Venkataramannagudem, West Godavari District. Selected F_2 plants were selfed and generated F_3 progeny which were evaluated during *kharif* 2021, at PG and Ph.D. Research Block, Department of vegetable science, College of Horticulture, Venkataramannagudem. The experimental site was well prepared, cultural practices include training, pruning, weeding, irrigation, fertilizer application and plant protection measures were followed for the healthy growth of crop. Observations were recorded on various yield parameters from all the plants of F_3 generation number of fruits per vine, fruit length (cm), fruit diameter (cm), average fruit weight (g), number of seeds per fruit, fruit yield per vine (kg), TSS (^{0} Brix) and Vitamin-C (mg/100g), GCV, PCV, Heritability analysis and Genetic Advance.

3. Results and Discussion

The mean, GCV, PCV, heritability, genetic advance as percent mean are given in Table 1, Table 2, Table 3, Table 4 and Table 5.

In the present investigation, the magnitude of GCV and PCV were closer in all the five crosses of F_3 generation *viz*. Pusa Sandesh × Arka Bahar, Pusa Sandesh × Punjab Bahar, Pusa Naveen × Pusa Santhusti, Pusa Naveen × Local Long, Pusa Naveen × Local Round for majority of the characters. This result suggests that, greater contribution of genotype rather than environment to the variability present in different traits. Similar findings were observed by Rashid *et al*. (2020), and Kandasamy *et al*. (2019) in bottle gourd, Kannan and Rajamanickam (2019) and Gautham and Balamohan (2018) in ridge gourd. The values of PCV were slightly higher than GCV which indicated the minor role of environment on the population in five crosses studied. These results were similar with the findings of Chandramouli *et al*. (2021) in bottle gourd and Deepa *et al*. (2018) in cucumber.

High estimates of GCV and PCV were observed in the traits *viz.*, number of fruits per vine, average fruit weight, fruit yield per plant in Pusa Sandesh × Arka Bahar, average fruit weight, fruit yield per plant, fruit length in Pusa Sandesh × Punjab Bahar, average fruit weight, number of seeds per fruit, fruit yield per plant in Pusa Naveen × Pusa Santhusti, fruit yield per plant in Pusa Naveen × Local Long, number of fruits per vine, fruit yield per plant in Pusa Naveen × Local Round. These results were indicating that there is a broad range of variability in the population and further selection in these traits play a major role. These results were in accordance with Chandramouli *et al*. (2021) in bottle gourd, Gautham and Balamohan (2018) and Kannan and Rajamanickam (2019) in ridge gourd.

Moderate estimates of GCV and PCV were observed in the traits *viz.*, fruit length, number of seeds per fruit in Pusa Sandesh × Arka Bahar, number of fruits per vine, fruit diameter in Pusa Sandesh × Punjab Bahar, number of fruits per vine in Pusa Naveen × Pusa Santhusti, number of fruits per vine, fruit length and average fruit weight in Pusa Naveen × Local Long, number of fruits per vine, fruit length, average fruit weight, number of seeds per fruit in Pusa Naveen × Local Round.

Table 1: Mean, GCV, PCV, heritability and genetic advance in F_3 population of Pusa Sandesh × Arka Bahar

Sl. No.	Character	Mean	GCV (%)	PCV (%)	h^2	GA	GAM (%)
1	Number of fruits per vine	11.14	24.53	26.46	85.92	5.22	46.83
2	Fruit length (cm)	34.86	9.12	11.82	59.50	5.05	14.49
3	Fruit diameter (cm)	31.57	5.30	9.25	62.75	1.99	22.47
4	Average fruit weight (g)	1181.43	26.18	28.95	81.77	566.22	48.77
5	Number of seeds per fruit	325.71	15.25	20.33	56.24	76.71	23.55
6	Fruit yield per vine (kg)	16.69	30.24	39.73	57.92	7.91	47.40
7	Total soluble solids (°B)	3.03	9.11	11.83	59.28	0.44	14.45
8	Vitamin-C (mg/100g)	8.29	4.57	5.52	68.43	0.65	7.79

Table 2: Mean, GCV, PCV, heritability and genetic advance in F_3 population of Pusa Sandesh × Punjab Bahar

Sl. No.	Character	Mean	GCV (%)	PCV (%)	h^2	GA	GAM (%)
1	Number of fruits per vine	16.29	17.17	19.06	81.14	5.19	31.86
2	Fruit length (cm)	14.43	17.80	25.06	66.44	1.59	10.38
3	Fruit diameter (cm)	22.14	16.41	19.93	67.82	6.17	27.85
4	Average fruit weight (g)	1337.86	20.86	22.70	84.42	528.19	39.48
5	Number of seeds per fruit	298.14	7.11	9.15	75.41	12.63	4.27
6	Fruit yield per vine (kg)	19.60	21.12	31.16	45.97	5.78	19.50
7	Total soluble solids (°B)	3.08	13.14	14.61	80.88	0.75	24.33
8	Vitamin-C (mg/100g)	7.61	6.09	8.26	54.30	0.70	9.24

Table 3: Mean, GCV, PCV, heritability and genetic advance in F_3 population of Pusa Naveen × Pusa Santhusti

Sl. No.	Character	Mean	GCV (%)	PCV (%)	h^2	GA	GAM (%)
1	Number of fruits per vine	7.43	13.46	20.17	44.55	1.37	18.51
2	Fruit length (cm)	32.43	8.06	9.16	47.55	4.74	14.63
3	Fruit diameter (cm)	34.39	8.68	10.31	60.42	3.13	9.10
4	Average fruit weight (g)	1168.59	26.13	32.23	65.71	509.86	43.63
5	Number of seeds per fruit	298.57	22.46	25.98	74.70	119.38	39.98
6	Fruit yield per vine (kg)	9.15	44.19	47.50	86.55	7.75	34.70
7	Total soluble solids (°B)	3.09	8.30	11.15	55.47	0.39	12.74
8	Vitamin-C (mg/100g)	9.57	5.77	6.20	86.54	1.06	11.06

Table 4: Mean, GCV, PCV, heritability and genetic advance in F_3 population of Pusa Naveen × Local Long

Sl. No.	Character	Mean	GCV (%)	PCV (%)	h^2	GA	GAM (%)
1	Number of fruits per vine	6.00	18.13	21.82	69.05	1.86	31.04
2	Fruit length (cm)	32.43	9.89	11.92	68.86	5.48	16.91
3	Fruit diameter (cm)	42.86	10.08	10.71	88.59	8.37	19.54
4	Average fruit weight (g)	1325.14	12.62	16.85	56.05	257.84	19.46
5	Number of seeds per fruit	292.86	9.73	14.42	45.52	35.59	13.52
6	Fruit yield per vine (kg)	8.87	18.63	25.56	63.08	2.48	27.95
7	Total soluble solids (°B)	2.85	10.45	13.00	64.61	0.49	17.30
8	Vitamin-C (mg/100g)	8.66	8.82	8.90	61.05	0.53	6.16

Table 5: Mean, GCV, PCV, heritability and genetic advance in F_3 population of Pusa Naveen × Local Round

Sl. No.	Character	Mean	GCV (%)	PCV (%)	h^2	GA	GAM (%)
1	Number of fruits per vine	9.00	23.11	25.20	87.13	3.93	33.67
2	Fruit length (cm)	18.00	16.72	21.00	63.43	4.94	27.44
3	Fruit diameter (cm)	43.74	6.13	6.92	40.57	1.80	4.11
4	Average fruit weight (g)	1427.29	11.72	16.77	68.87	241.00	16.89
5	Number of seeds per fruit	391.57	11.53	17.00	46.04	63.13	16.12
6	Fruit yield per vine (kg)	12.16	28.56	34.99	66.66	5.84	48.04
7	Total soluble solids (°B)	3.53	7.34	10.71	47.02	0.37	10.37
8	Vitamin-C (mg/100g)	8.17	7.47	8.54	76.53	1.11	13.53

It implies that moderate amount of variability is present in the population and further selection would be possible up to some extent. These results were in accordance with Janaranjani and Kanthaswamy (2015) in bottle gourd and Deepa *et al.* (2013) in cucumber.

Low estimates of GCV and PCV was observed in the traits *viz.*, fruit length, fruit diameter, TSS and vitamin-C in Pusa Sandesh × Arka Bahar, number of seeds per fruit, TSS and vitamin-C in Pusa Sandesh × Punjab Bahar, fruit length, fruit diameter, TSS and vitamin-C in Pusa Naveen × Pusa Santhusti, Pusa Naveen × Local Long and Pusa Naveen × Local Round. These characters would have less scope for exploitation in further generations. Similar results were obtained by Kanimozhi *et al.* (2015) in wax gourd.

High heritability coupled with high genetic advance was observed in the traits *viz.*, fruit length, fruit diameter, average fruit weight, number of seeds per fruit, fruit yield per plant in all the five crosses *i.e.*, Pusa Sandesh × Arka Bahar, Pusa Sandesh × Punjab Bahar, Pusa Naveen × Pusa Santhusti, Pusa Naveen × Local Long and Pusa Naveen × Local Round. This indicates the presence of additive gene action in inheritance of these traits. So, there was ample scope for direct selection in these traits. These results were similar with the findings of Chandramouli *et al.* (2021), Rashid *et al.* (2020), Kandasamy *et al.* (2019) in bottle gourd, Ramesh *et al.* (2018) in ridge gourd.

Moderate to high heritability coupled with low genetic advance was observed in the traits *viz.*, TSS and vitamin-C in all the five crosses *i.e.*, Pusa Sandesh × Arka Bahar, Pusa Sandesh × Punjab Bahar, Pusa Naveen × Pusa Santhusti, Pusa Naveen × Local Long and Pusa Naveen × Local Round. The high value of heritability accompanied with low genetic advance as per cent of mean indicated the non-additive gene action in inheritance of these traits. High heritability was due to high environmental influence rather than the genotype. Direct selection for such traits may not be rewarding. Similar results were obtained from the findings of Deepa *et al.* (2018) and Rani *et al.* (2017) in bottle gourd.

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Microbial analysis of jeevamrutha prepared from different cow breeds of desi and cross cow breeds cow dung and cow urine

Santosha Gowda G B[1], **K V Sudhir Kamath**[1] and **Lakshmana**[2]
[1]Diploma College of Agriculture, Brahmavar, Udupi
[2]Associate Director of Research, ZAHRS, Brahmavar, Udupi

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ABSTRACT

A laboratory experiment was conducted on shelf-life of jeevamrutha at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udupi. In this study three different desi breeds *viz*., Malnad gidda, Gir, Sahiwal and three different cross breeds *viz*., Holstein Friesian (HF), Jersey and crossbred Jersey, cow dung and cow urine were collected aseptically and separately to prepare Jeevamrutha, after preparation of Jeevamrutha from 1st day to 15th days samples were collected (daily) and enumerated the general microorganisms *viz*., bacteria, fungi and actinomycetes with their respective media. Among the desi and cross cow breeds jeevamrutha, desi cow breeds jeevamrutha contains higher microbial population compared to cross cow breeds jeevamrutha. In desi cow breeds jeevamrutha, Malnad gidda cow breed jeevamrutha contains the maximum microbial population. In general, the highest microbial population was noticed between 7th to 9th days after preparation (DAP) of jeevamrutha in all the cow breeds. Hence, it's considered as a best time for the application of jeevamrutha to soil to improve the soil organic carbon.

KEY WORDS: *Jeevamrutha*; *Organic carbon*; *Desi breeds*; *Cross breeds*

1. Introduction

The cost of inorganic fertilizers is increasing enormously to an extent that they are out of to reach small and marginal farmers. Use of inorganic fertilizers and insecticides, the population of beneficial organism's decrease and natural regeneration of nutrition in the soil cease (Rama and Naik, 2017; Dakshayini *et al*., 2016; Reddy *et al*., 2015). Soil becomes barren and soil fertility decreases. The use of fermented liquid manures in such situation is, therefore practically a paying proposal. Application of these organic liquid formulations will enhance the soil microbial activity and population to a larger extent. This in-turn has a positive effect on growth and yield of crops. Similarly, Subhash Palekar is one of the progressive farmers of Maharashtra, India; in his workshop on Philosophy and Technology of Zero Budget Natural Farming (ZBNF) he used a new biodynamic formulation termed jeevamrutha prepared from desi cow dung and cow urine. The desi cow or indigenous breed of cows is the backbone of ZBNF. For centuries, dung and urine from desi cows have been used in farming. Although the milk productivity of Indian cow breeds is low, they are very useful in production of cow dung and urine which will have a very

CONTACT Santosha Gowda G B santoshagowdagb@gmail.com

high beneficial property. According to Subhash Palekar, one gram of desi cow dung contains 300 to 500 crore beneficial effective microbes as against 50 to 70 lakh microbes in cross bred cow dung. Hence, Cross bred jersey and Holstein Friesian (HF) cows should not be used in ZBNF (Palekar, 2006). Vanaja *et al*. (2009) stated that jeevamrutha is a plant growth-promoting substance containing beneficial microorganisms that provides the necessary nutritional requirement for growth and yield of a crop.

Cow dung was used as major ingredient for the preparation of jeevamrutha. It contains numerous microorganisms; they are *Azotobacter*, *Acetobacter*, *Azospirillum* (nitrogen supplier), *Pseudomonas* (phosphorus-solubilizer) and *Bacillus silicus* (potash-solubilizer) and others. Once jeevamrutha was incorporated to soil, these organisms are well activated and maintain the soil productivity. Manjunatha *et al*. (2009) reported that the use of jeevamrutha (indigenous species cow dung and cow urine, pulse flour, jaggery, rhizosphere soil solution) treated organics, improves the physico-chemical and biological properties of soil (Arpitha and Dakshayini, 2024), besides improving the efficiency of applied farmyard manure. They also confirmed that the potential of jeevamrutha is to supply materials and to act as food support for beneficial microbes.

2. Material and Methods

A laboratory experiment was conducted at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udupi.

Three desi cow breeds (Malnad Gidda, Gir and Sahiwal) and three cross cow breeds (Holstein Friesian (HF), Jersey and cross Jersey) were selected for the experiment.

2.1 Preparation of Jeevamrutha

All the cow breeds cow dung and cow urine was collected aseptically and separately to prepare Jeevamrutha, A standard procedure was used to prepare Jeevamrutha (Palekar, 2006); 1.25 kg of cow dung, 1.25 lit. of cow urine, 250 g of pulse flour, 250 g of jaggery, one handful of soil and 25 lit. of tap water were used to prepare 25 lit. of jeevamrutha. All the ingredients were mixed in a plastic bucket; the mouth of the bucket was covered with gunny cloth and the bucket was kept in the room temperature for 15 days. Each day the content was mixed thoroughly with a wooden stick and the sample was collected in a sterile polythene bottle to analyse the microbial population.

2.2 Microbial analysis

The biological properties such as total microbial population of bacteria, fungi and actinomycetes were analysed (Rama *et al*., 2015). The method advocated for the enumeration was serial dilution and plate count technique with appropriate medium. Enumeration of microbial population was carried out using Nutrient agar for bacteria, Martin's Rose Bengal Agar (MRBA) for fungi, Actinomycetes selective media for actinomycetes at 10^6, 10^4 and 10^3 dilutions respectively and the plates were incubated at 28 ± 2 ºC.

2.3 Statistical analysis

The data obtained from experimentation were statistically analysed using completely randomized design (CRD). The statistical analysis was done by using WASP: 2.0 (Web Agri. Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php) and mean

were separated by Duncan Multiple Range Test (DMRT).

3. Results and Discussion

The total microbial population *viz*., bacteria, fungi and actinomycetes, were significantly influenced by different storage days (1st day after preparation to 15th days after preparation). The pronounced increase in microbial population during ageing is clearly evident from Table 1, 2 and 3.

The higher microbial population were noticed in desi cow breeds jeevamrutha compared to cross cow breeds jeevamrutha. In desi cow breeds, maximum bacterial population were noticed in Malnad Gidda breed jeevamrutha (90.33×10^6/ ml of jeevamrutha) and the next best was Gir breed jeevamrutha (79.33×10^6/ ml of jeevamrutha). Among cross cow breeds, higher bacterial population were recorded in HF cow breed jeevamrutha (20.66×10^6/ ml of jeevamrutha), at 7th DAP of jeevamrutha. The population was gradually increased in the middle of storage (1st DAP to 7th DAP) and further decreased gradually (8th DAP to 15th DAP) in jeevamrutha, similar trend was observed in fungal population (Table 1 and 2).

Actinomycetes population was maximum on 9th DAP of jeevamrutha in all the desi breeds, however jeevamrutha prepared with Malnad Gidda (20.33×10^3/ ml of jeevamrutha) recorded the highest population of Actinomycetes compared to all other desi breeds of jeevamrutha at 9th DAP (Table 3). Radha and Rao (2014) also reported the slow growth of actinomycetes in freshly prepared fermented liquid organic formulation compared to bacteria and fungi population. Devakumar *et al*. (2014) observed

Table 1: Bacterial population of Jeevamrutha prepared from dung and urine of different cow breeds

Days After Preparation	Bacterial population in Jeevamrutha (CFU × 10^6 per ml of Jeevamrutha)					
	Malnad Gidda	Gir	Sahiwal	HF	Jersey	Cross jersey
1	40.33^{h}	35.66^{i}	32.00^{jk}	10.33^{h}	9.66^{i}	7.66^{h}
2	48.33^{g}	38.00^{i}	33.00^{ij}	12.66^{g}	11.33^{h}	8.66^{g}
3	57.33^{f}	48.66^{h}	35.66^{hi}	13.66^{f}	12.66^{ef}	9.66^{e}
4	61.66^{e}	56.33^{g}	38.33^{h}	14.66^{e}	13.66^{d}	10.33^{d}
5	77.33^{bc}	62.33^{ef}	55.66^{d}	16.33^{d}	15.33^{c}	12.33^{c}
6	86.66^{a}	72.33^{b}	68.00^{b}	18.66^{b}	16.33^{b}	14.33^{b}
7	90.33^{a}	79.33^{a}	77.33^{a}	20.66^{a}	18.33^{a}	16.33^{a}
8	81.33^{b}	69.33^{bc}	62.33^{c}	18.66^{b}	15.33^{c}	12.33^{c}
9	73.33^{cd}	67.33^{cd}	51.00^{e}	17.66^{c}	13.33^{de}	10.33^{d}
10	70.00^{d}	64.33^{de}	49.33^{ef}	15.33^{e}	12.33^{fg}	9.33^{ef}
11	65.33^{e}	59.66^{fg}	47.00^{fg}	13.66^{f}	11.66^{gh}	9.00^{fg}
12	51.00^{g}	47.00^{h}	46.00^{g}	12.00^{g}	11.00^{h}	7.66^{h}
13	49.33^{g}	35.00^{i}	35.33^{hi}	10.66^{h}	9.66^{i}	6.33^{i}
14	29.33^{i}	29.66^{j}	29.00^{k}	10.33^{h}	9.33^{i}	6.00^{ij}
15	19.66^{j}	13.00^{k}	13.00^{l}	10.00^{h}	9.00^{i}	5.66^{j}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT).

Table 2: Fungi population of Jeevamrutha prepared from cow dung and cow urine of different cow breeds

Days After Preparation	Fungi population of Jeevamrutha (CFU × 10^4 per ml of Jeevamrutha)					
	Malnad Gidda	Gir	Sahiwal	HF	Jersey	Cross jersey
1	10.00^{j}	9.66^{h}	7.33^{h}	5.66^{j}	6.00^{h}	5.00^{l}
2	16.33^{i}	14.66^{f}	12.00^{g}	6.33^{ij}	7.66^{g}	6.33^{k}
3	20.66^{ef}	19.66^{d}	17.66^{e}	7.00^{hi}	8.00^{g}	7.00^{j}
4	25.66^{c}	23.66^{c}	22.66^{c}	8.00^{g}	10.00^{f}	7.66^{i}
5	27.66^{b}	26.66^{b}	25.66^{b}	9.66^{f}	11.33^{e}	8.33^{h}
6	29.33^{a}	28.33^{a}	27.33^{a}	10.00^{f}	12.00^{e}	10.00^{f}
7	30.66^{a}	29.33^{a}	26.33^{ab}	15.33^{b}	15.66^{c}	13.33^{c}
8	25.66^{c}	24.66^{c}	22.33^{c}	17.00^{a}	18.00^{b}	15.00^{b}
9	23.66^{d}	20.00^{d}	19.66^{d}	14.66^{b}	18.66^{ab}	15.66^{a}
10	22.00^{e}	18.33^{e}	17.66^{e}	13.00^{c}	19.00^{a}	13.66^{c}
11	19.00^{gh}	18.00^{e}	17.33^{e}	12.66^{cd}	15.66^{c}	12.00^{d}
12	19.33^{fg}	15.66^{f}	15.66^{f}	12.00^{d}	14.66^{d}	11.33^{e}
13	17.66^{hi}	12.33^{g}	11.66^{g}	11.00^{e}	11.66^{e}	9.00^{g}
14	11.00^{j}	7.66^{i}	7.33^{h}	8.00^{g}	10.00^{f}	7.66^{i}
15	9.66^{j}	5.33^{j}	4.66^{i}	7.66^{gh}	9.66^{f}	5.00^{l}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

Table 3: Actinomycetes population of Jeevamrutha prepared from dung and urine of different cow breeds

Days After Preparation	Actinomycetes population of Jeevamrutha (CFU × 10^3 per ml of Jeevamrutha)					
	Malnad Gidda	Gir	Sahiwal	HF	Jersey	Cross jersey
1	6.66^{i}	4.33^{k}	4.00^{i}	4.00^{g}	4.00^{hi}	1.00^{k}
2	8.66^{h}	6.33^{j}	4.66^{h}	5.00^{f}	4.66^{fg}	1.66^{j}
3	10.33^{g}	8.33^{h}	5.33^{g}	5.66^{e}	5.00^{f}	5.66^{g}
4	12.33^{f}	11.33^{f}	6.66^{de}	7.33^{d}	6.00^{e}	6.66^{f}
5	13.33^{e}	12.66^{e}	7.00^{d}	8.00^{c}	7.66^{c}	9.00^{e}
6	14.00^{e}	13.66^{d}	9.00^{b}	9.66^{a}	9.33^{ab}	11.66^{c}
7	16.66^{c}	15.33^{c}	9.33^{ab}	10.00^{a}	9.66^{a}	11.33^{c}
8	19.33^{b}	18.33^{b}	9.66^{a}	8.66^{b}	9.00^{b}	13.66^{b}
9	20.33^{a}	19.33^{a}	7.66^{c}	7.00^{d}	7.00^{d}	15.00^{a}
10	15.66^{d}	13.66^{d}	6.33^{ef}	6.00^{e}	6.66^{d}	10.00^{d}
11	13.66^{e}	13.33^{de}	6.00^{f}	5.66^{e}	6.00^{e}	6.33^{f}
12	10.33^{g}	10.00^{g}	5.00^{gh}	4.00^{g}	4.33^{gh}	5.33^{g}
13	8.66^{h}	7.33^{i}	4.66^{h}	2.66^{h}	3.66^{ij}	4.33^{h}
14	8.33^{h}	6.66^{ij}	3.00^{j}	2.00^{i}	3.33^{j}	4.66^{h}
15	5.33^{j}	4.33^{k}	1.66^{k}	1.66^{i}	2.00^{k}	3.66^{i}

Note: Means with same superscript, in a column do not differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT)

higher colony forming units of bacteria, actinomycetes, fungi and nitrogen fixers in Jeevamrutham at 7th DAP. Babu (2011) reported that uncountable rate of *Bacillus*. The higher microbial population of these liquid organic formulations made them as a potent source to maintain soil fertility and to enhance the nutrient availability by helping in faster decomposition of bulky organic manures (Kumar *et al*., 2023; Shilpa *et al*., 2015).

The over-all results reviewed that; the highest microbial population were observed between 7th to 9th days after preparation of jeevamrutha. Hence, it's considered as a best time for the application of jeevamrutha, out of six cow breeds, jeevamrutha prepared with Malnad Gidda showed maximum microbial population compared to other cow breeds jeevamrutha. These microbes help to improve the plant growth by different mechanisms such as fixing of atmospheric nitrogen, solubilization of unavailable form phosphorus, potassium, zinc, organic matter decomposition *etc*., and also improve the soil fertility by increase with soil organic carbon.

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Potential of *Crotalaria grahamiana* and *Crotalaria spectabilis* as previous crop and intercrop against bacterial wilt (*Ralstonia solanacearum*) of rain-fed potato (*Solanum tuberosum* L.) in the Highlands of Madagascar

Lovaniaina Dinanirina Andriamiarisoa[1], **Tendro Radanielina**[2], **Nirina Rakotoarimanga**[3] and **Volatsara Baholy Rahetlah**[4]

[1]Plant Protection Direction, Antananarivo, Madagascar
[2]Department of Plant Biology and Ecology, Faculty of Sciences, University of Antananarivo, Madagascar
[3]National Research Centre for the Environment, Antananarivo, Madagascar
[4]Department of Agroecology, Biodiversity and Climate change, Ecole Supérieure des Sciences Agronomiques, University of Antananarivo, Madagascar

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ABSTRACT

Bacterial wilt caused by the soil-borne species complex *Ralstonia solanacearum* is a serious disease affecting potato (*Solanum tuberosum* L.) yield and quality in tropical, subtropical and warm temperate regions. In Madagascar, bacterial wilt occurs in all potato production areas causing severe crop losses and posing a serious threat to rural households' food security. The present study carried out under rain-fed, field conditions at two sites located, in the Betafo district, in the Vakinankaratra region, the main potato production area aimed to assess biological control potential of two legume cover crops (*Crotalaria spectabilis*; *Crotalaria grahamiana*) as previous crop and intercrop against potato bacterial wilt. The experiment conducted on a moderately susceptible potato variety (Meva) on andosol was laid out in randomized complete block design with four treatments and four replicates. Treatments were consisted of potato sole cropping (control), potato mulching and intercropping with *C. grahamiana*, potato green manuring and intercropping with *C. grahamiana* and potato intercropping with *Crotalaria spectabilis*, respectively. Results showed positive effects of intercropping systems on potato yield parameters and biocontrol effects against potato bacterial wilt caused by strains of *R. solanacearum* phylotype I, biovar 3. Delayed bacterial wilt onset, decreased bacterial wilt incidence (-38 to -75%) and decreased infected tubers rates (-61 to -96%) were recorded in Crotalaria mixed cropping as well as increased potato total tubers (+107 to 145%) and marketable tubers (+125 to +170%) yields.

KEY WORDS: *Bacterial wilt*; *Crotalaria spp.*; *Biological control*; *Potato*; *Ralstonia solanacearum*

1. Introduction

Bacterial wilt caused by the soil-borne plant pathogenic species complex *Ralstonia solanacearum* is a serious disease of potato in tropical, subtropical and warm temperate regions

CONTACT Volatsara Baholy Rahetlah rahetlahvolatsara@gmail.com

(Hayward, 1991). The *Ralstonia solanacearum* species complex has broad plant host range comprising more than 200 species belonging to over 50 botanical families. They are responsible of several types of bacterial wilt disease on economic groups and ornamentals worldwide (Elphinstone, 2005).

Traditionally, the *Ralstonia solanacearum* species complex strains were classified into five (5) races based on their phenotypical properties, their differences in host range and into 5 biovars based on their differential ability to utilize carbon source from three hexose alcohols and three disaccharides (Hayward., 1994). Race 1 regroups strains that affect tobacco, potato, tomato, eggplant, diploid banana and many other Solanaceous crops and weeds. Race 2 strains affect triploid banana, plantain causing Moko disease as well as the ornamental plants, species of *Heliconia* spp. Race 3 strains mainly cause wilt on potato and tomato under temperate conditions. Race 4 strains and race 5 strains are specific to ginger and mulberry, respectively.

The *Ralstonia solanacearum* species complexe's genetic diversity has been recently distributed into four phylogenetic groups (phylotypes) that correspond to their origin based on phylogenetic analysis of sequence data generated from 16S-23S ribosomal inter-genic spacer region as follows: Phylotype I, strains originating from Asia; Phylotype II, strains from the Americas; Phylotype III, strains from Africa and Phylotype IV, strains from Indonesia (Fegan and Prior, 2005). The phylotypes are subdivided into sequevar groups based on sequence variation in the hrpB and endoglucanase (egl) partial genes (Fegan and Prior, 2005).

The vascular pathogen penetrates host through roots natural openings such as emerging points of secondary roots and through nematode or insect-induced wounds (Vasse *et al*., 1995). Following penetration, the pathogen colonize and multiply massively in the xylem vessel, producing abundant exopolysaccharides before progressing into other parts of the pant. The production of exopolysaccharides combined with callose deposition from host, as a response to the infection, ultimately lead to xylem vessels obstruction and characteristic wilting symptoms development.

On potato, *Ralstonia solanacearum* strains cause wilting of aboveground vegetative parts and rotting of tubers at harvest and storage. Some strains can survive latently in potato tubers without triggering symptoms (Ciampi *et al*., 1981). The main source of inoculum are infected plant, contaminated soil, contaminated irrigation and surface water, latent infected potato seed tubers (Kelman *et al*., 1994).

In Madagascar, potato is an important food crops in the central Highlands, especially in the Vakinankaratra region, the main production region where area under potato production accounts more than 50 000 ha. In the Vakinankaratra region, potato is playing a key role in household food security as rice supplement or substitute during the lean period. In addition, potato is high value commercial crops generating supplementary incomes (CEFFEL, 2012).

Bacterial wilt is one of the most widespread, severe diseases affecting potato yield and quality and posing serious threat to rural households' food security. Over the last decade, potato bacterial wilt outbreaks have been reported as more severe and more frequent.

Like other soil-borne disease, bacterial wilt is difficult to manage. No conventional method effectively controls it. Sub-groups of the species complex *Ralstonia solanacearum* encompasses strains that can survive for extended periods in soil and in roots and rhizosphere of many hosts (reservoirs) including weeds (Prior *et al*., 1990). Recent trends in bacterial wilt control are mainly focused on integrative measures including sustainable, ecological-friendly cultural practices based on sanitizing crops. These methods mostly rely upon enhancing host plant's resistance and promoting soil health through decreasing infectious potential of soil and modifying soil microbial communities for disease suppression.

The present field study aimed at assessing biological control potential of two legume (Papilionaceae), sanitizing crops *Crotalaria grahamiana*, *Crotalaria spectabilis*, against potato bacterial wilt. *Crotalaria grahamiana*, a perennial species, is generally opportunistic, widespread throughout the Highlands and *Crotalaria spectabilis*, an annual species that is geographically restricted to the Midwestern part of the country. Both Crotalaria species are widely used in farming systems as green manure crop, cover crop and hedgerow crop.

2. Materials and Methods

2.1 Experimental sites

The present field study was carried out at two sites located at the rural Commune of Mandritsara (19°49'39" S; 46°53'19" E, 1,576 masl) and the rural Commune of Alakamisyanativato (19°53'01" S; 46°54'20" E, 1524 masl), in the Betafo District, in the Vakinankaratra region, in the Highlands of Madagascar, over one rainy season, from November 2020 to May 2021.

The climate is altitude tropical with a dry and cool season extending from May to September with minimal daily temperature ranging from 6°C to 9°C and a rainy and warm season extending from October to April with average daily temperature ranging from 13.2°C to 26°C. Mean annual rainfall averages exceed 1300 mm (Ahmim-Richard *et al*., 2018).

Soil type at the two sites are andosol, acidic or moderately acidic, relatively rich in carbon and in nitrogen, with a satisfactory C/N ratio, moderately rich in phosphorus and in potassium, with a loamy sand texture (**Table 1**).

2.2 Treatments and experimental design

The perennial (*Crotalaria grahamiana*) and the annual (*Crotalaria spectabilis*) herbaceous legume cover crops and the Meva potato cultivar, moderately susceptible to bacterial wilt caused by *Ralstonia solanacearum* were used for the field experiment. This potato cultivar is particularly appreciated by potato growers owing to its agronomic traits (earliness, 150 days of growth, fair storability), its technological characteristics (higher dry matter content, suitability to frying)

Table 1: Soil chemical properties at the experimental sites

Site	pH	C%	N%	C/N	P (Bray II) ppm	K (meq/100g)
Mandritsara	4.82	4.20	0.270	13.9	3.50	0.209
Alakamisyanativato	5.26	6.54	0.423	15.5	6.00	0.216

Source : Laboratoire de Radio Isotope, Madagascar

and its higher economic value on domestic markets.

The experimental design was a randomized complete block design with four treatments and four replicates. Treatments were consisted of T_1: potato sole cropping, T_2: potato mulching and intercropping with *C. grahamiana*, T_3: potato green manuring and intercropping with *C. grahamiana*, T_4: potato intercropping with *C. spectabilis*. Each of the elementary plots contained 40 plants of potato planted at 0.7 × 0.3 m rows, under standard fertilizer composed of 10 t ha^{-1} of farmyard (cattle) manure and 33 kg N + 66 kg P_2O_5 + 48 kg K_2O ha^{-1} in the form of NPK complex 11-22-16 applied at potato planting time complemented with additional 46 kg N ha^{-1} in the form of urea applied four weeks after potato planting during earthing up time (15-20 cm).

Under intercropping system, *Crotalaria* sp. seeds were sown in alternate rows (0.7×0.3m), in drill sowing pattern, 12 weeks prior to potato planting (previous cropping). Six weeks after sowing, *C. grahamiana* aerial biomass was entirely cut down and either allowed to die back to form a mulch (T_2) or incorporated into the soil (T_3) four weeks before potato planting while the root systems remain in the soil.

2.3 Sampling, measurement and analysis

Potato yield parameters measurement

At harvest (twelve weeks after planting), potato yield parameters [total number and yield of tubers per plant; total number and yield of commercial tubers (≥ 28 mm diameter) per plant] were measured on 12 potato plants located in the central two rows.

Bacterial wilt incidence monitoring

In each plot, bacterial wilt incidence was monitored at 5, 6, 7 and 8 weeks after potato planting using the formula:

Incidence (100%) = Σ wilted plants × 100 / total plants

At harvest, infected potato tubers percentages for each plot were calculated.

Isolation and characterization of presumptive Ralstonia solanacearum strains

Bacterial strains were isolated from stem, tuber vascular tissue of diseased potato plants.

Serial dilutions from stem, tuber vascular tissue and soil samples were spread onto semi-selective medium (SMSA: modified Sequiera Medium South Africa) (Elphinstone *et al.*, 1996) and onto the triphenyltetrazolium chloride (TZC) medium.

Presumptive colonies of *R. solanacearum* developing the typical white with pink centres, irregularly-shaped, fluidal morphotype were subcultured and purified on TZC medium. Pure bacterial isolates were, subjected to biochemical characterization (biovar determination) and species and Phylotype-levels molecular characterization.

Biochemical characterization or biovar determination aimed at testing the ability of presumptive *R. solanacearum* strains to produce acids on Ayers basal media amended with three hexose alcohols (mannitol, sorbitol, dulcitol) and three disaccharides (cellobiose, lactose and maltose) as carbon source (French *et al.*, 1993). Molecular characterization was carried out at species and phylotype level using reference strains.

DNA extraction from presumptive isolates of R. solanacearum

A loopful (1µl) of presumptive RSSC single colonies from a CPG plate was suspended in 100µl molecular biology grade water and boiled at 95°C for 15 min on a thermal cycler for DNA extraction (Weller *et al*., 2000).

PCR detection of RSSC and phylotype identification

DNAs from the boiled presumptive *Ralstonia* isolates are first screened using RSSC-specific primer pairs 759/760 of Opina *et al*. (1997). Phylotypes are screened by multiplex PCR combining the four phylotype-specific primers: N: mult21:1F (phylotype I), N: mult21:2F (phylotype II), N:mult23:AF (phylotype III), N:mult22:InF (phylotype IV) and the species-specific reverse primer Nmult22:RR as described by Fegan and Prior (2005).

The PCR is run with the following cycling conditions: initial denaturation of 15 min at 95°C; 30 cycles of 30 s at 94°C, 1 min at 59°C and 1 min at 72°C and final extension of 10 min at 72°C. The PCR reaction reagents, volumes and protocol were as described by Abdurahman *et al*. (2019). PCR products were visualized through electrophoresis, after application of an electric current of 90 volts for 40 minutes.

Statistical analysis

Para-metric data were subjected to analysis of variance followed by means separation according to the Tukey's honestly significant difference test ($p<0.05$). For non-parametric data, Kruskal-Wallis test ($\alpha = 0.05$) and then a pairwise comparison test was performed ($\alpha = 0.05$) using the software XLSTAT. Data in percentages were transformed into logarithmic (Log 10) data prior to analysis of variance.

3. Results and Discussion

Biovar determination, species confirmation and phylotype identification

Biochemical tests revealed that all presumptive strains of *Ralstonia solanacearum* isolated from stem, tuber vascular tissue of symptomatic potato plants were capable of using all disaccharides and all hexose alcohols except inositol as carbon source.

Therefore, they can be classified into biovar 3. Molecular characterization carried out at species and phylotype level revealed 280bp fragment and 144bp fragment, respectively (**Fig. 1** and **2**). *Ralstonia solanacearum* strains belong to Phylotype I.

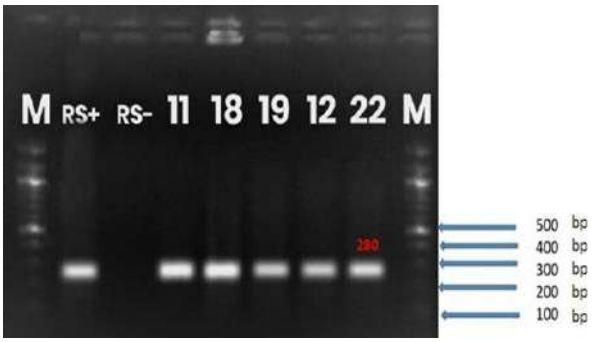

Fig.1: Gel electrophoresis of PCR amplification products

Lane M: DNA Marker; lane RS+: reference strain of R. solanacearum ; lane RS- :negative control, lane 18 : positive sample of R. solanacearum isolated from Alakamisyanativato, Betafo; 22: positive sample isolated from Mandritsara, Betafo

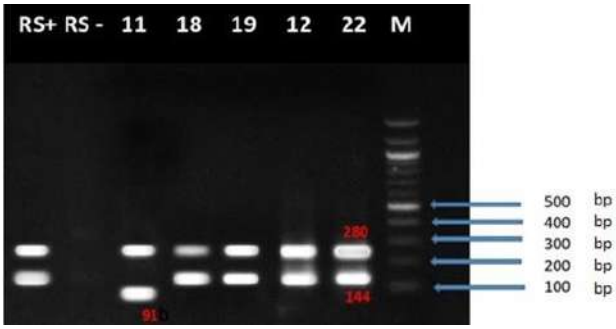

Fig. 2: Gel electrophoresis of Multiplex PCR amplification products

Lane M: DNA Marker; lane RS+: reference strain of R. solanacearum ; lane RS- :negative control, lane 18 : positive sample of R. solanacearum isolated from Alakamisyanativato, Betafo; 22: positive sample isolated from Mandritsara, Betafo

Potato agronomic parameters

At harvest, twelve (12) weeks after planting, potato total tubers and marketable yields per plant were significantly different between sites, treatments and their interactions. Over the two sites, intercropping systems gave +107 to +145% total potato tubers yield advantage over potato sole cropping. The highest yield advantage was recorded for soil treatment under mulching and intercropping with *Crotalaria grahamiana* (**Fig. 3**).

With regards to potato marketable tubers yield per plant, they are significantly ($p<0.05$) different between sites, treatments and their interactions. Over the two sites, maximum potato marketable tubers yield advantage was recorded for soil treatment under mulching and intercropping with *Crotalaria grahamiana*. Average potato marketable tubers fresh weight was +125 to +170% superior under intercropping systems (**Fig. 3**)

Potato bacterial wilt incidence

Bacterial wilt incidence was recorded from 5 to 8 weeks (37 to 56 days) after potato planting.

At both sites, bacterial wilt incidence increased over the time. Under intercropping system with

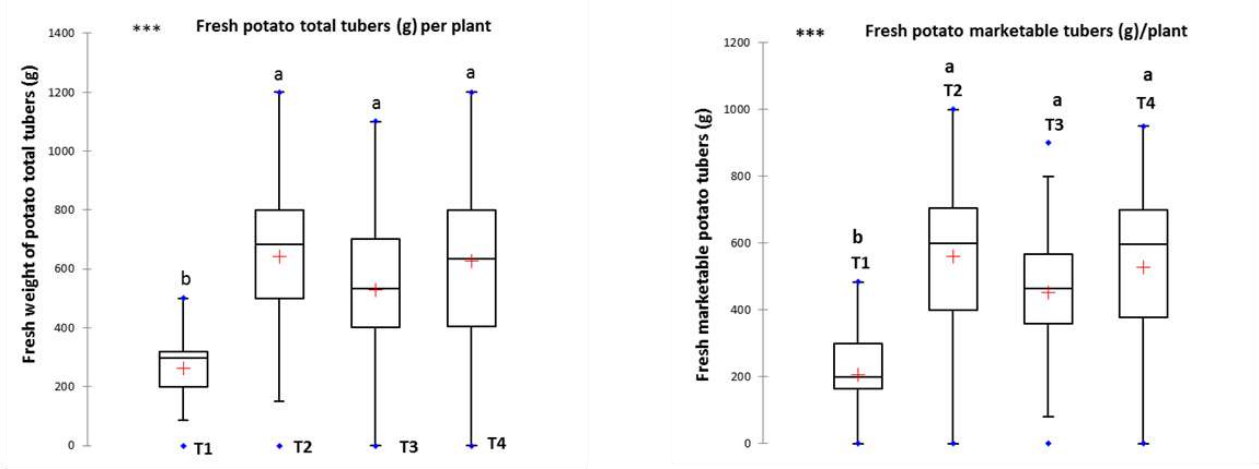

Fig. 3: Potato total tubers yield (left) and marketable tubers yield (right)

(T_1: potato sole cropping, T_2: potato mulching and intercropping with *Crotalaria grahamiana*, T_3: potato green manuring and intercropping with *Crotalaria grahamiana*, T_4: potato intercropping with *Crotalaria spectabilis*, within columns means followed by the same letter are not significantly different at $p = 0.05$ according to the Kruskel-Wallis all pairwise comparison test)

Crotalaria spectabilis, bacterial wilt symptoms onset was delayed about one week at the Alakamisyanativato site where the legume cover crops grew rapidly. At 8 weeks after potato planting, cumulative bacteria wilt incidence varied significantly ($p<0.05$) between sites, treatments and their interaction. Over the two sites, bacterial wilt incidence was significantly declined by -38 to -75%, the lowest incidence was recorded for intercropping system with *Crotalaria spectabilis* (**Table 2**).

At harvest, bacterial wilt infected potato tubers percentage was decreased by -84 to -91% and -61 to -96% under soil treatment with mulching and intercropping of *Crotalaria grahamiana* and soil treatment with intercropping of *Crotalaria spectabilis* as compared to potato sole cropping, at the Mandritsara site and the Alakamisyanativato site, respectively. Over the two sites, averaged potato bacterial wilt incidence was -76 to -93% lower (**Table 2**).

Intercropping of moderately susceptible cultivar Meva of potato either with annual legume cover crops *Crotalaria spectabilis* or perennial legume cover crops *Crotalaria grahamiana*, on andosol showed potential to promote potato growth and control of bacterial wilt caused by strains belonging to phylotype I and biovar 3 of the species complexe *Ralstonia solanacearum*. Potato yield parameters in terms of total and marketable potato tubers yields were positively affected in significant way by intercropping.

Potato growth stimulation can be associated with positive effects of intercropping on soil physico-chemical properties leading to improved uptake of nutrients.

Positive effects of potato intercropping with

Table 2: Cumulative potato bacterial wilt (BW) incidence and infected tubers

Treatment	BW incidence (log %)			Potato tubers infection (Log %)		
	Mandritsara	Alakamisyana tivato	Mean	Mandritsara	Alakamisyana tivato	Mean
T_1 (control)	1.03 ± 0.05 a	1.10 ± 0.02 a	1.06 ± 0.05 a	1.12 ± 0,10 a	8.47 ± 5.97 a	0.90 ± 0.48 a
T_2	0.85 ± 0.13 ab	0.67 ± 0.58 ab	0.76 ± 0.39 ab	0.20 ± 0.35 b	0.38 ± 0.38 a	0.18 ± 0.28 b
T_3	0.94 ± 0.06 ab	0.63 ± 0.54 ab	0.78 ± 0.39 ab	0.27 ± 0.46ab	3.28 ± 1.66 a	0.36 ± 0.40ab
T_4	0.65 ± 0.16 b	0.20 ± 0.17 b	4.17 ± 0.12 b	0.19 ± 0.33 b	1.68 ± 1.68 a	0.10 ± 0.23 b
p-value (site)	-	-	0.09	-	-	ns
p-value (treatment)	0.043	0.049	0.018	0.024	ns	0.005
p-value (site×treatment)	-	-	0.49	-	-	ns
Mean	0.87 ± 0.17 b	0.65 ± 0.48	0.76 ± 0.37	0.44 ± 0.50	3.45 ± 5.74	0.39 ± 0.47

T_1: potato sole cropping, T_2: potato mulching and intercropping with *Crotalaria grahamiana*, T_3: potato green manuring and intercropping with *Crotalaria grahamiana*, T_4: potato intercropping with *Crotalaria spectabilis*, within columns means followed by the same letter are not significantly different at $p = 0.05$ according to the Kruskel-Wallis all pairwise comparison test

legume cover crops were reported by other researchers (Guitari *et al*., 2018). Field experiment carried out in Kenya on rainfed potato intercropped with lima bean and dolichos revealed benefits of intercropping such as enhanced radiation interception and use efficiency, soil temperature, water content and productivity optimization. However, results obtained from the present experiment revealed that intercropping of potato with *Crotalaria* spp., particularly with *Crotalaria spectabilis* has great potential to control potato bacterial wilt through delayed disease onset, reduced incidence of bacterial wilt on potato aerial plant part (-75%) and tubers (-96%) (Nyawade *et al*., 2019).

Similar results were observed from a field experiment carried out in bacterial wilt endemically-infested field in Uganda, aiming at testing the effect of *Crotalaria falcata* as fallow and potato intercrop. Significant reduction of potato bacterial wilt incidence by 85% was recorded under soil fallowing with *Crotalaria falcata* and reduction of infected potato tubers by more than 94%. Reduced bacterial wilt incidence was coupled with suppression of latent R solanacearum infection after 6 to 12 months potato intercropping with *C. falcate* (Kakuhenzire *et al*., 2013).

In addition, results from a field experiment carried out in Martinique reported potato bacterial wilt onset delay of 25 days and reduction of bacterial wilt incidence up to 70% through previous cropping of *C. spectabilis* and *C. juncea* (Deberdt *et al*., 2015).

Besides having soil physico-chemical properties improving effects, *Crotalaria* spp. have suppressive effects on plant parasitic nematodes (Wang *et al*., 2002). They are generally poor host or non-host to many plant parasitic nematodes producing secondary metabolites, allelochemicals (pyrrolizidine) toxic to nematodes and bacteria (Joosten and Van Veen, 2011). Moreover, Crotalaria species are able to stimulate nematode-antagonistic microorganisms, hence preventing bacterial wilt infection through nematode-induced wounds. Other studies reported that soil organic matter increase was correlated with growth of facultative bacteria which compete with *R. solanacearum* strains (Cardoso *et al*., 2006). However, Vanitha *et al*. (2012) revealed that *Crotalaria grahamiana* exhibit phenolic compounds (flavonoids) in its different parts with significant antimicrobial activity.

Biological control effects of *Crotalaria spectabili*s and *Crotalaria grahamiana* against bacterial wilt can be associated with various mechanism such as potato nutritional quality and disease resistance enhancement through improved soil chemical and physical properties, or soil disease suppression through either diversity and composition of soil microbial communities modification or R. solanacearum specific antagonistic strains stimulation (Cardoso *et al*., 2006). Bacterial wilt symptoms apparition and intensity are dependent on several factors including host characteristics (species, cultivar and physiological stage), *R. solanacearum* strain type, level of inoculum and interactions with the environment (temperature, humidity, type of soil) (Van *et al*., 2004).

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The economic importance of effective Rhizobial nodulating and yield of Soybean (*Glycine max* L.) at Assosa Western Ethiopia

Bakala Anbessa[1] and **Zerihun Getachew**[1]
[1]Ethiopia Institute of Agricultural Research, Asossa Agricultural Research Center, Assosa, Ethiopia

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ABSTRACT

A field experiment was conducted on Nitisols of Asossa Agricultural Research Centre during the 2016/17 to 2017/2018 cropping season to investigate the effect of rhizobia nodulation on yield and yield components of soybean. The rhizobia and inorganic phosphorous fertilizer treatments considered in the study consisted of three rhizobia strains and three rhizobia strains plus phosphorous fertilizer including one. The treatments consist of negative control (T1), rhizobia MAR-1495-SB, (T2), rhizobia SB12 (T3), TAL 379 (T4), rhizobia MAR-1495-SB+50kg DAP (T5), rhizobia SB12+50kg DAP (T6) and Rhizobia TAL 379 +50kg DAP (T7). The treatments were laid out as a Randomized Complete Block Design with three replications. The analysis of variance revealed that rhizobia had non-significantly ($P > 0.05$) affected grain yield. The maximum (2187.9 kg ha^{-1}) grain yield was recorded from rhizobia MAR-1495-SB bio-fertilizer at Asossa district. The application of MAR-1495-SB rhizobia biofertilizer had the highest net benefit of 26,094.8 ETB, followed by rhizobia MAR-1495-SB+50kg DAP biofertilizer which also had a total of 23,518.6 ETB net benefit at Asossa district. The application of MAR-1495-SB rhizobia bio-fertilizer had the highest net benefit. Therefore, we recommended the treatment MAR-1495-SB rhizobia since it produced a high marginal rate of return, high net benefit and relatively small total cost of production, for soybean production in the Asossa area.

KEY WORDS: *Rhizobia*; *Inoculant*; *Strain*; *Net Benefit*; *Marginal rate of return*

1. Introduction

The Leguminosae is one of the most important and largest plant families and is composed of about 750 genera containing 16,000–19,000 species distributed worldwide. Leguminosae has major impacts on agriculture, the environment, animal/human nutrition, and health, of which soybean is one of the world's most important and miraculous pulse crops. It accounts for 29.7% of the world's processed vegetable oil and is rich in dietary protein both for human food and animal feed (Graham and Vance, 2003). In Ethiopia, the area allocated for soybean and the corresponding total annual production has been 31,876 ha and 63,653 tons, respectively, with a productivity less than 2 ton ha^{-1} (CSA, 2012), while the potential soybean yield has been estimated to be in the range of 6 – 8 tons ha^{-1} in USA (Cooper, 2003). Biological nitrogen fixation (BNF) and mineral soil or N fertilizers are the main sources for meeting the N requirement of high-yielding soybean varieties. BNF is an effective and efficient source of N supply to plants under

CONTACT Bakala Anbessa anbessa2004@gmail.com

favorable atmospheric and environmental conditions (Chen *et al*., 2002). More than 50–83% of the necessary N requirement for soybean can be derived from BNF (Schipanski *et al*., 2010) by symbiotic association with either the genus *Bradyrhizobium* or *Sinorhizobium*. Several research findings clearly showed that soil nitrate repressed nodulation and the effect was magnified as soil nitrate concentrations increased (Hungria *et al*., 2006). BNF is very useful for smallholder farmers as it is cost-effective, environmentally friendly, meets the N requirement of the legumes and reduces the N demand of the succeeding crops. Inoculation with compatible and effective rhizobia may be necessary to optimize the nitrogen fixation and hence legume grain yields, where a low population of native rhizobial strains predominates (Mastrodomenico and Purcell, 2012). Therefore, evaluation and identification of appropriate and effective rhizobial strains are crucial to enhance nitrogen fixation and yield of soybean.

In the present investigation, therefore, the influences of soybean maturities group and effectiveness of *Bradyrhizobium* spp., in soil with high N and having no rhizobial association with soybean were thoroughly examined under greenhouse and field conditions using drip irrigation. This research work hypothesized that high soil N decreases the effectiveness of *Bradyrhizobium* sp. inoculation in medium-maturing soybean genotypes but may not be observed with late-maturing soybean genotypes. Therefore, the specific objective of this piece of research work was to evaluate the influence of symbiotic effectiveness of isolates of *Bradyrhizobium* spp on the soybean at the nitosol of Asossa.

2. Material and Methods

2.1 Description of the study area

The study was conducted at the Assosa Agricultural Research Center which is located in Assosa District in the Benishangul-Gumuz Regional State. The Benishangul-Gumuz Regional State is located in the western part of Ethiopia between 9°30' to 11° 39" N and 34° 20' to 36° 30" E covering a total land area of 50,000 square kilometers (km^2). The Assosa District is characterized by hot to warm moist lowland plain with a uni-modal rainfall pattern. The rainy season starts at the end of April and lasts at the end of October with a maximum of June, July, August and September. The total annual average (2007-2014) rainfall is 1316 mm. The annual mean minimum and mean maximum temperatures of the District for the periods from 2007 to 2014 are 16.75 and 27.92 °C, respectively.

2.2 Experimental treatments and design

The treatments consisted of six rhizobial strains (Strain MAR-1495, Strain SB12, TAL 379, Strain MAR-1495 + 50kg TSP, Strain SB12+50kg TSP, TAL 379 +50kg TSP) and control (without any fertilizer). The experiment was laid out as a randomized complete block design (RCBD) with a replicated three times. The plot size was 3m×4m and the spacing of between rows and between plants 60cm and 5cm, respectively. The blocks were separated by a 1.5m wide open space whereas the plots within a block were separated by a 0.75m wide space. Soil bunds were constructed around each plot and around the entire experimental field to minimize nutrient, water movement, and cross-contamination from plot to plot. Weed control was achieved manually by hand picking. Crop growth was then monitored until harvest.

2.3 Soil sampling and preparation

Before the field experimentation, ten random samples (0-20m depth) were collected and composite soil samples were prepared. These composite samples were used for soil physical and chemical analysis. Similarly, post-crop harvest soil samples were collected plot-wise from each replication from the surface 0-20 cm depth for selected soil chemical analysis. The soil samples were air dried, sieved to pass through a 2 mm sieve, and placed in labeled plastic bags.

2.4 Sources of seeds and inoculum

The soybean genotype used for this study was provided by the Asossa Agricultural Research Center, Ethiopia, which has been approved to be superior under Asossa field conditions. One soybean genotype, which was late maturing (Belsa 95), was used for the field experiment. Rhizobial isolates, namely *Bradyrhizobium japonicum* (TAL-379 isolate), MAR 1495 isolate and SB12 isolate were used as inoculants. These isolates were obtained from Holleta Agricultural Research Center (UK- isolate) and National Soil Research Center in Addis Ababa (TAL-379 isolate).

2.5 Plant data collection and analysis

Central row plants were used for data collection. Growth-indicating parameters such as plant height, number of seeds per pod number of pods per plant and grain yield were collected. The plant height (cm) was measured from the base of the plant to upper the top most leaves of the plant. The data was taken from five randomly selected plants a few days after the fully filled seed. Five plants from the central rows were randomly taken to count the number of nodules per plant. The number of pod per plant and number of seed per pod was computed from five plants. For the number of seeds per pod, five plants and from each five randomly selected seed per pod was recorded. Finally, after threshing the soybean, the grain yield from five plants was recorded and grain yield per hectare was calculated. The grain yield was adjusted at 11.5% grain moisture content.

2.6 Partial budget analysis

The mean grain yield of the selected treatment was used in the partial budget analysis (CIMMYT, 1988). Economic analysis was performed to investigate the economic feasibility of the treatments (fertilizer rates). A partial budget, dominance and marginal analysis were used. The average open market price (Birr kg^{-1}) for ground nut and the official prices of Urea and biofertilizers were used for economic analysis. The dominance analysis procedure as detailed in CIMMYT (1988) was used to select potentially profitable treatments from the range that was tested. The selected and discarded treatments using this technique are referred to as undominated and dominated treatments, respectively. The undominated treatments were ranked from the lowest (the farmers' practice) to the highest-cost treatment. For each pair of ranked treatments, a percent marginal rate of return (MRR) was calculated. The percent MRR between any pair of undominated treatments denotes the return per unit of investment in fertilizer expressed as a percentage.

2.7 Statistical data analysis

Analyses of variances for the data were recorded and conducted using the SAS GLM procedure (SAS 1998). Least significant difference (LSD) test at 5% probability used for mean separation when the analyses of variance indicate the presence of significant differences.

3. Results and Discussion

3.1 Soil Physical and Chemical Properties

According to the laboratory analysis, the soil texture of the experimental area is clay (Table 1). Maize usually grows well under good soil conditions. A fertile, medium textured, sandy or clay loam and alluvial soils of good fertility with optimum soil pH of 5.5 to 7 soil usually is best (Barrow, 2017). The pH of the soil is 6.2, which is moderately acidic. According to Sharma *et al*. (2015), this value is considered to be a low pH value. At low pH values, phosphate ions combine with iron and aluminum to form compounds, which are not readily available to plants.

However, Miller and Donahue (1997) indicated that plants grow well between pH 5.5 and pH 8.5. Maize response to applied P depends on soil acidity, soil OM level and clay content. Clay content affects the interpretation of soil test values obtained by extraction, and values for clay soils will likely be very different from those for sandy soils. Therefore, P fertilizer recommendations will depend on soil texture (Abdulaziz, 2013). The CEC of the soil of the experimental site is 22.6 cmol kg^{-1} of soil. According to Landon (1984), this value lays in the lower range (15-25 cmol kg^{-1}), which means the soil is not satisfactory for agricultural production. Further, the analysis indicated that the experimental soil has values of 0.29%, 2.46%, 11.5 ppm and 0.1443 mill-equivalents/100g soil for total N, OM, available P, and exchangeable K, respectively (Table 1). When the results of the analysis are compared with the broad ratings made by Metson (1961), the values lie in the lower range for plant growth except for total N and available phosphorous.

3.2 Seed yield of soybean and yield components

Analysis variance of two locations revealed that non-significant difference ($P < 0.05$) due to the application of treatments for the means of seed yield. Mean grain yield was non-significantly ($P < 0.05$), however, enhanced by 55.7% with the application of strain MAR1495, 24.5 % with the application of strain TAL365 and 18.1 with the application of strain SB12 over zero-strain or -ve control (Table 2). However, there was no significant difference observed between strains

Table 1: Major soil chemical characteristics of the experimental site

Sl. No.	Soil Character	Values	Remark
1	Soil pH (by 1:2.5 soil water ratio)	6.2	Moderately Acidic
2	Total Nitrogen (%)	0.29	High
3	Organic matter content (%)	2.46	Moderate
4	Available phosphorous (ppm)	11.5	High
5	Cation exchange capacity (cmol (+) kg^{-1})	22.6	Low
6	Exchangeable potassium (meq/100 g soil)	0.1443	Very Low
7	Soil texture		
	Clay (%)	59.4	
	Sand (%)	30.5	
	Silt (%)	10.1	
	Textural class	Clay	

Table 2: Evaluation of strains on yield and major yield determinant parameters of soybean at Asossa zone of Benshal-gul Gumuz

Treatments	PH (cm)	PPP	SPP	GY (kg)
Control	68.2	18.9	2.5	1404.6
Strain MAR-1495	64.9	21.5	2.5	2187.9
Strain SB 12	66.7	22.4	2.4	1714.1
TAL 379	68.2	20.7	2.5	1748.5
Strain MAR-1495 + 50 kg DAP ha^{-1}	67.3	19.6	2.4	2025.3
Strain SB 12+ 50 kg DAP ha^{-1}	65	19.1	2.5	1764.8
TAL 379 + 50 kg DAP ha^{-1}	68.4	20.3	2.5	1705.1
LSD	8.3	6.7	0.3	584.1
F-test	NS	NS	NS	NS
CV%	7	18.5	7.37	18.3

**= P<0.01, *=P<0.05 and NS = Non Significant at P>0.05, PH = Plant height, PPP = Number of pod per plant, SPP = Number of seed per pod, GY = Grain yield

MAR1495, SB12, TAL365 and zero strain.

This study is non-consistent with the result of Rugheim and Abdelgani (2012), who reported that inoculation of rhizobia strains significantly increased faba bean yield. Desta *et al*. (2015) also confirmed that application of effective rhizobia strains alone and/or in combination with zinc significantly increases faba bean yield. The report by Youseif *et al*., 2017 also shows that application of effective strains increases faba bean grain yield by up to 44-47%. The highest grain yield of 2187.9 kg ha^{-1} was recorded from the plot that received strain MAR1495 and it was at par with Strain MAR-1495 + 50 kg DAP ha^{-1}. The lowest grain yield of 1404.6 kg ha^{-1} was recorded in from a zero strain plot or plot that no received any things. Antenah (2014) has also described that, soybean plants treated with the UK isolate inoculation produced the highest total biomass yield, exceeding the total biomass yield produced by plants in the control treatment by about 47.3%. In line with Tahir *et al.* (2009) reported that combined application of rhizobia inoculation and phosphorous application resulted in 21% increased grain yield.

Based on the present findings, it can be concluded that despite the availability of adequate soil N, symbiotic N does, indeed, increase yield in late-maturing soybean genotypes. Treatment Strain SB 12+ 50 kg DAP ha^{-1} had the lowest number of pods per plant among inoculation treatments, but this was not significantly (P>0.05) different from Strain MAR-1495, Strain SB 12, TAL 379, Strain MAR-1495 + 50 kg DAP ha^{-1}, TAL 379 + 50 kg DAP ha^{-1} and the control (Table 2). Treatment Strain SB 12 had the largest number of pods per plant, but this was comparable with yields of treatments Strain MAR-1495, TAL 379, Strain MAR-1495 + 50 kg DAP ha^{-1}, TAL 379 + 50 kg DAP ha^{-1} (Table 2).

3.3 Partial budget analysis

The increased production of the crop due to the application of inputs might or might not be beneficiary to farmers (CIMMYT, 1988).

Therefore, partial budget analysis (CIMMYT, 1988) was employed to estimate the net benefit, dominance analysis and marginal rate of return that could be obtained from various alternative treatments (CIMMYT, 1988). The MAR 1495-SB rhizobia had the highest net-benefit of 26,094.8 Ethiopian birr, followed by Strain MAR-1495-SB + 50 kg DAP ha^{-1} rhizobia which also had a total of 23,518.6 Ethiopian birr net benefit. The lowest net benefit was obtained by the application of the Negative control and TAL 379 inoculant + 50 kg DAP ha^{-1} with net benefit of 16,855.2 and 19,676.2 ETB the respectively (Table 3).

The profitability of the study showed that application of MAR 1495-SB rhizobia which provided the relatively highest net benefit (26,094.8 ETB), was recommended to apply bio fertilizers. The highest net benefits from the application of inputs for the production of the crop might not be sufficient for the farmers to accept as good practices. In most cases, farmers prefer the highest profit (with low cost and high income). For this purpose it is necessary to conduct dominated treatment analysis (CIMMITY, 1988).

The dominance analysis showed that the net benefits of all treatments were dominated except application of MAR 1495-SB and MAR-1495-SB + 50 kg DAP ha^{-1}. The % MRR between any pair of undominated treatments denotes the return per unit of investment in fertilizer expressed as a percentage. Economic analysis revealed that maximum marginal rate of return was recorded with application of MAR 1495-SB (5774.5). The marginal rate of this treatment was well above the 100% minimum (CIMMYT, 1988). Accordingly, the study revealed that application of MAR 1495-SB was considered as the best for recommendation. The best recommendation for treatments subjected to marginal rate of return is not necessarily based on the highest marginal rate of return, rather based on the minimum acceptable marginal rate of return and the treatment with the highest net benefit, relatively low variable cost together with an acceptable MRR becomes the tentative recommendation (CIMMYT, 1988).

4. Conclusion

In recent years, crop productivity in Ethiopia in general and in Benshal-gul Gumuz region in

Table 3: Economic and Partial budget analysis of bio-fertilizer and inorganic fertilizer

Treatments	GY (kg)	VC	TGR (ETB ha^{-1})	NB (ETB ha^{-1})	MRR%
Control	1404.6	0	16,855.2	16,855.2	0
Strain MAR-1495-SB	2187.9	160	26,254.8	26,094.8	5774.5
Strain SB 12	1714.1	160	20,569.2	20,409.2	D
TAL 379	1748.5	160	20,982	20,822.0	D
Strain MAR-1495-SB + 50 kg DAP ha^{-1}	2025.3	785	24,303.6	23,518.6	497.5
Strain SB 12+ 50 kg DAP ha^{-1}	1764.8	785	21,177.6	20,392.6	D
TAL 379 + 50 kg DAP ha^{-1}	1705.1	785	20,461.2	19,676.2	D

Note: Prices: Urea= 8.24 birr kg^{-1}, TSP=12.75 birr kg^{-1}, Price of soybean=12 birr kg^{-1}, Seed=15 birr $kg^{-1}$and Labor cost =30 birr/ person/day for 8 hours, TC=Total cost, Gross return (Return from Grain) = Price /kg* yield in kg and Net return = Gross return – Total cost, VC = Variable cost, GR= Growth return, TGR = Total growth return from grain, NB = Net benefit

particular has shown a declining trend, in spite of the best use of improved varieties. The most possible causes of this decline soil fertility depletion and the continuous use of the traditional fertilizer, which have limited the yield and crop quality. Therefore this experiment was designed for the purpose of evaluated the rhizobial strain types and inorganic P for soybean under field condition of Asossa District. The rhizobia strain on nodulation parameters had highly significant difference ($P< 0.001$), however there was no significant differences ($p>0.05$) between the rhizobia strain and rhizobia strain plus inorganic phosphorus fertilizer on grain yield. Accordingly, the study revealed that application of Strain MAR-1495 as the best strain recommended agronomical for soybean production at Assosa area. For all parameters the Strain MAR-1495 rhizobial strain alone increased yield and yield components as compared with 1495 + 50 kg DAP ha^{-1}. A substantial increase in nodulation directly affected growth and yield due to the N_2 fixation potential of soybean. Application of rhizobium inoculation alone also increased nodulation, growth and yield of soybean because of high ppm-P (phosphorous availability) of the study area during experimentation. The profitability of the study showed that MAR 1495-SB strain which provided the relatively highest net benefit (26,094.8 ETB), was the best strain for Asossa district. The best recommendation for treatments subjected to marginal rate of return is not necessarily based on the highest marginal rate of return, rather based on the minimum acceptable marginal rate of return and the treatment with the high net benefit, relatively low variable cost together with an acceptable MRR becomes the tentative recommendation. Therefore economically we recommend the treatments MAR 1495-SB that have acceptable marginal rate of return, relatively high net benefit and relatively small total cost of production for soybean production at Asossa district. It can be recommended to demonstrate Strain MAR-1495 rhizobial strain to increase productivity and sustainability of soybean for study area and similar agro-ecology with its area.

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Overview of an emerging pest in Rice: *Leptispa pygmaea* Baly (Coleoptera: Chrysomelidae)

Krishna Japur
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad

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ABSTRACT

Leptispa pygmaea Baly, commonly known as the rice blue beetle, is an emerging pest that poses a significant threat to rice production worldwide. This review paper provides a comprehensive overview of *L. pygmaea*, including its taxonomy, distribution, life cycle, feeding habits, economic impact, and management strategies. The goal is to enhance the understanding of this pest and facilitate the development of effective management approaches to mitigate its damage. Through an in-depth analysis of available literature and research studies, this review highlights the urgent need for integrated pest management strategies that encompass cultural, biological, and chemical control methods to effectively combat the spread of *L. pygmaea*

KEY WORDS: *Leptispa*; *Coleoptera*; *Rice*; *Chrysomelidae*

1. Introduction

Rice, *Oryza sativa* is the second most important cereal crop after wheat in the world and is the most important grain with regard to human nutrition and caloric intake. It is a staple food crop for more than two third of the population of India and more than 65% of the world's population (Mathur *et al*., 1999). Rice is probably the world's most genetically diverse crop, which thrives well under varying ecosystems starting from rainfed upland (dry systems) to rainfed lowland (wet system) and in deep water situations (Rajehja, 1995).

In India, rice occupies an area of 43.95 million hectares with annual production of 106.54 million tonnes and productivity is 2.42 tonnes/ha (Anonymous, 2015). Though the production is large, the per hectare yield is very poor as compared to other rice growing countries like Spain, Japan, Australia and China. The main reasons for low productivity are vagaries of nature, low fertilizer use efficiency, poor management of insect-pests and heavy infestation of weeds.

Rice is affected by more than 100 insects, among which 10-12 pose an economic threat to rice cultivation worldwide and decrease the productivity. The emerging threat of *L. pygmaea*, commonly decrease rice production is a cause for concern in agricultural communities. This pest primarily affects rice crops by feeding on the leaves, causing significant damage.

CONTACT Krishna Japur krishnauasd@gmail.com

2. Taxonomy and Distribution

The rice blue beetle belongs to the following taxonomic hierarchy position;

Kingdom : Animalia (Animals)
Phylum : Arthropoda (Arthropods)
Class : Insecta (Insects)
Order : Coleoptera (Beetles)
Suborder : Polyphaga
Superfamily : Chrysomeloidea
Family : Chrysomelidae (Leaf beetles)
Genus : *Leptispa* Baly, 1858
Species : *L. pygmaea*

2.1 Genetic structure and phylogeny status of *L. pygmaea*

The Polymerase Chain Reaction (PCR) targeting the COI gene fragment of *L pygmaea* resulted in the amplification of a single product measuring 695 base pairs (bp) in length. The evolutionary history of *L. pygmaea* was inferred using the Neighbor-Joining method. Phylogenetically *Colasposoma* sp., *Neolochmea dilatipenni and Chelymorpha alternans* are the nearest relative of *L pygmaea* (Mashhoor *et al*., 2013).

Leptispa pygmaea, has a global distribution, although it is native to Southeast Asia. Over time, it has spread to various regions around the world. Here are some details about its distribution and spread patterns. The blue beetle is the emerging insect pests of rice in India in recent times, the first record of rice blue beetle, *L. pygmaea* Baly from Assam and West Bengal by (Maulik, 1919) and its distribution were in Assam, Kerala, Karnataka, Maharashtra, Meghalaya and West Bengal states of India and Elsewhere in Sri Lanka (Anonymous, 1999). This pest is also known to occur in other Asian countries *viz*., Nepal, China, Ceylon, Vietnam (Sprecher, 1997), Bangladesh (APPPC, 1987), Pakistan (Fray, 1976) and Butan (Shinsaku, 2005). *L. pygmaea* was first reported as pests of paddy by Burlow (1899) and Lefroy (1906). *L. pygmaea* earlier considered as minor pest (Trehan, 1946; Patel and Patel, 1970; David and Kumaraswami, 1975 and Dale 1994).

Southeast Asia: It is indigenous to Southeast Asian countries such as Thailand, Malaysia, Indonesia, and the Philippines. It is believed to have originated in this region.

Africa: The beetle has been reported in several African countries, including Nigeria, Cameroon, Sudan, and Madagascar. It is considered an introduced pest in these areas, likely brought through trade or natural migration.

Australia: It has been detected in northern parts of Australia, including Queensland and the Northern Territory. The exact mode of its arrival in Australia is not clear, but it may have been introduced through international travel or trade.

Pacific Islands: The beetle has also been found in Pacific Island nations such as Papua New Guinea, Fiji, Solomon Islands, and Vanuatu. Its presence in these regions is likely due to human activities and the movement of agricultural commodities.

The spread patterns of *L. pygmaea* are influenced by multiple factors, including global trade, transportation and the expansion of rice cultivation. Infested plant materials, contaminated machinery, or accidental transportation by humans can facilitate the dispersal of the pest to new areas. Climate change and shifts in temperature and rainfall patterns may also influence its distribution and population dynamics.

3. Life cycle and Biology

The grub and adult blue beetles prefer young transplanted rice crop. The beetle is dark metallic blue in colour. Sexual dimorphism is observed between male and female beetles by their size. Male beetles are larger than females. The female beetles lay yellowish oval single eggs or in batch both on upper and lower surface of paddy leaves. The grub period has five larval instars and is more voracious feeders followed by female and male beetles. The severe grub feeding causes inward rolling of rice leaves often confused with the attack of leaf folder. The first four instars are yellowish green coloured and turns white before pupation. The pupa exhibits a brown coloration and is loosely attached to the leaf by its posterior end (Fig. 1).

The adult beetle damages by scrapping of chlorophyll material on the leaf surface which looks parallel streaks in appearance. The beetle damage is more in *kharif* than rabi season. There are no varieties/Hybrids completely resistant to this pest. Both varieties/cultivars from KAU and all the tested National entries under NSN2 from DRR were not completely resistant to this beetle. 'Jyothi' and 'Abhilasha' are the most preferred and high yielding rice variety of Kerala and Karnataka respectively. The beetle can be controlled by spraying any contact chemical insecticides, but control becomes difficult during *kharif* due to continuous south west monsoon showers. The beetle was not attracted to light traps.

Adult female lays oval shaped eggs on both the sides of leaf surface either singly or in parallel rows with an average fecundity of 12.67 eggs having 0.36 mm length and 0.16 mm width (Fig. 2). The incubation period was 4.5 days. The grub undergoes five larval instars, with an average developmental period of 10.9 days. The grubs display a range of sizes, measuring between 2.48 to 4.53 mm in length and 0.69 to 1.14 mm in width. The head size of the grubs ranges from 0.15 to 0.26 mm. The grub pupated on leaf surface by getting attached loosely with its posterior end. The pupal stage lasts for a period of 4.40 days, during which the pupa measures 3.71 mm in length and 1.17 mm in width. The entire life cycle of the pest is completed in 19.80 days. The longevity of adult beetle varied with sex and male beetles lived longer than female beetles. Adult male of *L. pygmaea* lived for 37 days with size of 6.81 mm in length and 2.08 mm width and female lived for 19.96 days with size of 6.20 mm length and 1.80 mm width (Fig. 2), whereas head size of both male and females measured 0.27 mm (Krishna *et al*., 2013b).

*Egg***:** *L. pygmaea* female beetle lays smooth pale yellow or pale green elliptical eggs in a straight line in batches of two, three to four eggs mostly on the lower surface of the leaf with 90.96-98.81% hatching (Patel and Patel, 1970; Dalvi *et al.*, 1985; Patel and Shah, 1985). The mean incubation period was 3.79 to 7.16 days. The females oviposited clutches of upto 8 eggs mostly on the adaxial side of the leaf (Kaniyarikkal *et al.*, 2009). The female *L. pygmaea* lays about 11-16 yellowish coloured eggs/ batch which were oval shaped on both the upper and lower surface of leaf and the grub hatched within 3-4 days (Karthikeyan and Sasomma, 2008a) and eggs hatch in 4-5 days with 0.35 to 0.38 mm length and 0.10 to 0.21 mm width (Krishna *et al*., 2013b).

*Larva***:** *L. pygmaea* larvae is a soft bodied campodeiform grub, dorso-ventrally compressed

and dirty white having a sclerotized tubular process at the abdominal tip. It had three larval instars. The first instar was completely white except head, which was brownish in colour immediately after emergence then later changed its colour to dirty white after taking food with an average period of 3.04 days. The larvae of second and third instars were dirty white in colour with duration of 3.33 and 4.97 days, respectively. The total larval period was 13.77 days in *kharif* season as against 13.22 days in off-season (Patel and Shah, 1985). The larvae arranged themselves in 7 a longitudinal line on the leaf surface, to a maximum of 10-12 with a mean of 7 per leaf (Dalvi *et al*., 1985 and Swamiappan *et al*., 1990). The grub had five larval instars each with duration of 1-2 days and completed grub development with a mean period of 8.2 days (Karthikeyan and Sosamma, 2008a). The grubs have five larval instars with mean developmental period of 10.9 days (Krishna *et al*., 2013b).

*First instar***:** The neonate grub was completely white except head which is brown in colour. After taking food the colour of grub changed to dirty white. It measured from 2.40 to 2.55 mm in length. The width of grub was 0.58 to 0.80 mm and the head width was 0.13 to 0.17 mm. The average period of this instar was 2.2 to 3.1 days.

*Second instar***:** The colour of second instar grub was dirty white and it measured from 3.50 to 3.90 mm in length, 0.90 to 1.12 mm in width and head width was 0.18 to 0.21 mm. Here second instar ranged from 1.80 to 2.70 days.

*Third instar***:** The third instar grub measured from 3.90 to 4.10 mm in length. The width of grub measures from 1.10 to 1.21 mm and the head width was 0.21 to 0.24 mm, with dirty white colour and the grub of this instar was able to move faster when disturbed. The grub instar ranged from 1.50 to 2.30 days.

*Fourth instar***:** The fourth instar grub with dirty white colour measured from 4.10 to 4.40 mm in length, 1.00 to 1.15 mm in width and head width was 0.24 to 0.27 mm. Here the grub instar ranged from 1.6 to 2.1 days.

*Fifth instar***:** The grub colour of fifth instar was also dirty white in colour and was measured from 4.40 to 4.60 mm in length, 1.00 to 1.18 mm in width and head width was 0.25 to 0.28 mm. In this instar the grub period was ranged from 2.00 to 2.40 days (Fig. 3).

*Pre-pupa***:** *L. pygmaea* did not spin a cocoon before pupation. Just before completion of pre-pupal stage, a drop of sticky anal fluid oozed out which helped in sticking the caudal ventral of the pre-pupa with the leaf and the pupa was duly formed in pre-pupal body and came out by splitting epicranial suture of the pre-pupa. The pre-pupal skin was completely removed by peristaltic movement within 23 to 27 minutes. The exuviae thus removed were retained folded on the leaf surface (Patel and Shah, 1985).

*Pupa***:** The pupa of *L. pygmaea* is milky white in colour when freshly formed, which changes subsequently to brown colour within a few minutes. The pupa is exarate type, brown coloured and attached itself to leaf surface by its posterior end. About three pale brown pupae were seen on each leaf. Appendages *viz*., head, antennae, mouthparts, wings and legs of developing adult could be seen through the pupal skin. Pupal stage lasted for 4-5 days. The total pupal period is 4.52 days during crop season as against 4.39 days during off-season.

A large number of white pupal skin was seen on leaf surfaces after beetle emergence (Patel and Patel, 1970; Dalvi *et al*., 1985; Patel and Shah, 1985; Swamiappan *et al*., 1990; Krishna *et al*., 2013b). Whereas, the pupal period was 3.2 and 2.9 days in Jyothi and Aishwarya varieties respectively and completed the life cycle within 14.8 in Jyothi and 13.8 days in Aishwarya (Karthikeyan and Sosamma, 2008a). The grub pupated on the surface of the leaf as a brown colour pupa and was seen newly formed pupa was white in colour which changed its colour to brown within few minutes. The pupa measured from 3.61 to 3.80 mm in length and width measured from 1.06 to 1.28. The pupal period was ranged from 3.9 to 5.1 days (Krishna *et al*., 2013b).

*Adults***:** Wings and abdomen of the newly emerged adults were completely white in colour (Fig. 4 and 5), after one to two hours of emergence the colour of wings changed to metallic bluish green in colour (Krishna *et al*., 2013b). The adult *L. pygmaea* is narrow, elongate and cylindrical with a slightly constriction at the centre. The beetle is deep metallic blue or dark greenish blue or dark bluish green with fine striations or small pitting on the elytra with more or less parallel rows of punctures. Elytra were finely striated at the extreme apex and also slightly reflexed to the dorsal side. The underside of the body was entirely black with 8-minute whitish hairs on it (Kadam *et al*., 1956; Patel and Shah, 1985; Dalvi *et al*., 1985; Swamiappan *et al*., 1990; Karthikeyan and Sosamma, 2008a).

The adult *L. pygmaea* displays a metallic greenish-yellow coloration and possesses longer antennae, a narrow thorax, and a lengthy body. On the other hand, the female rice blue beetle can be distinguished by its shorter antennae, broader thorax, and more robust body. The antennal scape will be broader in the female as compared to that of the male (Karthikeyan and Sosamma, 2012). Male beetles are bigger in size than females. The body length measured from 6.60 to 7.10 mm in males and 5.80 to 6.50 mm in females. The body width measured from 1.98 to 2.15 mm in males and 1.70 to 1.95 mm in females. The head width measured from 0.27 mm in both males and females. The longevity of adult varied with the sex of rice blue beetle. Male beetles lived longer than females. The life span of male beetles was ranged from 35 to 41 days and in females it was ranged from 18 to 23 days (Krishna *et al*., 2013b).

3.1 Copulation, pre-oviposition and oviposition period and fecundity

The mating behaviour of adults was found immediately after emergence from pupae and the copulation was found to be lasted for 5 to 10 minutes. The pre-oviposition period was ranged from 1.40 to 2.00 days and oviposition period was ranged from 4 to 6 days. The fecundity of *L. pygmaea* ranged from 9 to16 eggs/female on rice plants at sirsi, Karnataka (Krishna *et al*., 2013b).

L. pygmaea female laid 38-66 eggs on rice in konkan region of Maharashtra (Dalvi *et al*., 1985) while the fecundity ranged from 43.0-58.8 eggs in south Gujarat (Patel and Shah, 1985). During August and September months there was a rapid buildup of the pest due to congenial condition and number of eggs were more during this period (70-120 eggs / 5 hills) than the rest of the year (0-55 eggs / 5 hills). No egg laying was observed from the second week of October in the rice field of South Gujarat (Patel and Shah, 1985). Adult beetle laid oval shaped yellow eggs on both the leaf surfaces either singly or parallel rows with an

average fecundity of 16.8 and 14.3 eggs on short duration variety Jyothi and medium duration Aishwarya variety respectively, during June to October 2005 (Karthikeyan and Sosamma, 2008a).

Total life cycle: *L. pygmaea* has completes its life cycle with different developmental stages *viz*., egg, grub and pupa with a mean period of 4.50, 10.90 and 4.40 days, respectively and total life cycle completes in 19.80 days (Krishna *et al*., 2013b).

Adult's behaviour: The adults are very weak fliers (Swamiappan *et al*. 1990). The mating takes immediately after emergence of adults from pupae (Dalvi *et al*., 1985). Adults being polygamous mates throughout the day, but the active mating period are only during morning and evening hours. It also mates at night when adults were exposed to artificial light. Duration of coitus lasts for 4-8 minutes. Average periods for pre-oviposition, oviposition and post-oviposition were 4.91, 13.81 and 0.45 days, respectively (Patel and Shah, 1985). The female and male beetles were differentiated by the body size and type of antennae. Males were slightly smaller than females. In female beetle, the antenna was serrated with six uniform sized basal segments and four larger terminal segments while in males, the first basal segment was larger than the remaining segments with a remarkable serrate nature. Six segments in the middle were similar to each other and more or less of the same size. The terminal four segments were similar to each other and larger than the middle six segments (Patel and shah, 1985). The number of female beetles under field condition were less compared to males, with a sex ratio of 1:1.55 (♀: ♂) (Patel and Shah, 1985; Karthikeyan and Sosamma, 2008a).

4. Feeding habits and Damage

The adult beetles feed on rice leaves either by making holes or completely stripping the plant (Lefroy, 1906). Beetle completes its immature stages on the leaf surface and not as a leaf miner (Fletcher, 1913). Both larvae and adult feeds on the upper surface by scraping chlorophyll matter of rice leaves by making longitudinal white streaks on them (Dalvi *et al*. 1985). In the case of severe damage, the rice leaves were folded longitudinally and dried. As a result, the plants became very weak and dried up. From a distance, the rice field showed severely dried appearance. When the young crop was attacked, it resulted in stunting and severe drying symptoms. Incidence was found to be higher in shaded areas (Patel and Shah, 1985; Swamiappan *et al*., 1990). Neonate larvae of rice blue beetle migrates to the base of leaf axil and feeds by scraping, which induced formation of leaf rolls from the base, but adult feeds on the adaxial side of tender rice leaves which induced partial upward rolling of the leaf lamina Kaniyarikkal *et al*. (2009). Both the grubs and adult's feeds on the rice leaves by scraping the chlorophyll content in between the veins and veinlets which leads to streaks on them. The streaks made by grubs were shorter and narrower as compared to those done by adults (Krishna *et al*., 2013b).

5. Economic impact

In the recent past it is reported to cause pest outbreaks which are much concern in rice cultivation area of Kerala (Northern Districts of Palakkad, Kannur and Kasaragod), Karnataka (coastal region of UK district), Maharashtra (Konkan region) and Gujarat (Navsari district),

Fig 1: Damage symptoms of *L. pygmaea*

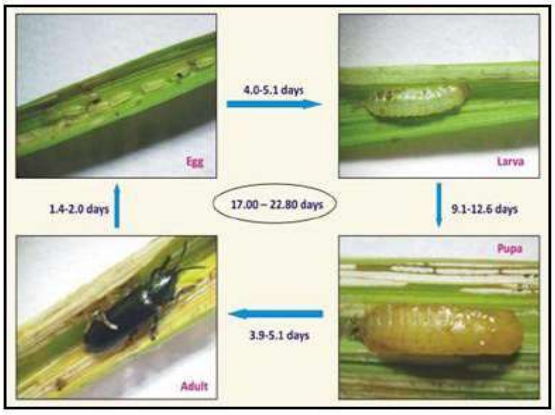

Fig 2: Biology of Rice blue beetle, *L. pygmaea*

Fig 3: Biology of Rice blue beetle, *L. pygmaea*

Fig 4: Dorsal and ventral view of pre-adult

Fig 5: Newly emerged adult with white wings

Fig 6: Dorsal and Ventral view of adult (♂ &♀) beetle

which is known to cause great loss in rice production. Weather factors, with a dominant role on the survivability, development and reproductive capacity of insect pests and exert a great influence on their population dynamics.

L. pygmaea has appeared in epidemic form for the first time during *Kharif* 1978 and since then the severe losses in localised pockets were reported from different parts of Konkan region every year in the state of Maharashtra. There were two peaks incidence of *L. pygmaea i.e.,* August to September (1st peak) and March to April (2nd peak) in Konkan region of Maharashtra. No egg laying and further development was observed in winter on alternate host plants during off season but it resumes its activity by January end on potted rice plant. Adults survived for 55 to 70 days on these food plants till next crop (Dalvi *et al*., 1985).

The severe outbreak of rice blue beetle occurred in kuzhithurai area of Kanyakumari District, Tamil Nadu during Nov-Dec 1988 and incidence was very severe on an area of about 50 ha of Ponmani variety during maximum tillering and panicle initiation stages (Swamiappan *et al*., 1990). *L. pygmaea* which was a minor pest of rice has recently been noticed to assume a serious status causing great concern to cultivars in several major rice growing tracts of Kerala state in India (Dale 1994; Karthikeyan and Sosamma, 2008c). The damage caused by *L. pygmaea* was more during early stage of crop especially during 1996-97 to 1998-99 (2.2 to 3.6 beetles/ hills) at Sirsi and Mundgod taluks of Uttara Kannada district (Prasad, 2003). The mean population of rice blue beetle (grubs and adults/ hill) under different rice ecosystem of Uttara Kannada district in upghat drill sown, upghat transplanted area and coastal transplanted area was (2.10, 0.92 and 0.65 respectively) (Rajendraprasad *et al*., 2011) and (6.19, 5.40 and 4.65 respectively) (Krishna *et al*., 2014). The rice blue beetle incidence started in Navasari district of Gujarat from 4th week after transplanting (WAT) during *Kharif* season reached peak level during 7th WAT with population of 3.01 beetle/ plant and 3.93% leaves damage whereas during summer it reaches peak level at 10th WAT with 0.55 beetle/ plant and 1.19% leaves damage (Patel *et al*., 2013).

The highest damage of blue beetle at early tillering stage was noticed in Jyothi (31.5 to 45.7%) and Aiswarya (19.5 to 29.5%) varieties under direct seeded condition. In Jyothi, 68.5 to 75.3% and Aiswarya 36.2 to 46.1% damage was noticed under transplanted crop (Karthikeyan and Sosamma, 2009a).

In India increase in blue beetle population often coincides with the transplanting of rice seedlings. The beetle leaves their hibernating sites by late May and start laying eggs by early June and oviposition continues till July end. Then the adults bury themselves in the debris and under the roots of grasses (Kuwayama, 1966). Cloudy condition with warm weather and frequent drizzling rain favours the buildup of *L. pygmaea* in the field during vegetative stage. The peak activity of rice blue beetle was observed during cooler hours of the day *viz*., early morning and late evening hours in the field and took shelter under shady portion of the plants during sunny period of the day. During the off-season, specifically from November to February, the pest manages to survive in its adult stage by residing on grasses, volunteer rice plants, ratoon rice, or sugarcane. It should be noted that the pest's level of activity is significantly reduced during this particular period. No egg laying has

been observed on the alternate hosts during the pests' inactive stage (Khanvikar *et al*., 1983).

The rice blue beetle incidence was the highest noticed in fourth week of August (14.2 beetles/hill) with 20.1% leaf damage, whereas the lowest damage was in July 1st week (6.6 beetles/hill) with 11.6% leaf damage (Krishna *et al*., 2013a). A strong negative correlation exists between the population of beetles and both grain

Table 1: Alternate hosts of rice blue beetle, *Leptispa pygmaea*

Sl. No	Common name	Botanical name	Family	Author
1	Globe finger rush	*Fimbristylis miliacea*	Cyperceae	Karthikeyan and Sosamma, 2009a
2	Pickerel weed	*Monochoria vaginalis*	Pontederiaceae	Karthikeyan and Sosamma, 2009a
3	Yellow sawah lettuce	*Limnocharis flava*	Limnocharitaceae	Karthikeyan and Sosamma, 2009a
4	Water hyacinth/ Lilac devil	*Eicchornia crassipes*	Limnocharitaceae	Karthikeyan and Sosamma, 2009a
5	Blistering ammania	*Ammania baccifera*	Lythraceae	Karthikeyan and Sosamma, 2009a
6	Torpedo grass	*Panicum repens*	Poaceae	Karthikeyan and Sosamma, 2009a
7	Wild rice	*Oryza rufipogon*	Poaceae	Karthikeyan and Sosamma, 2009a
8	Not known	*Isachne miliacea*	Poaceae	Karthikeyan and Sosamma, 2009a
9	Cupscale grass	*Sacciolepis interrupta*	Poaceae	Karthikeyan and Sosamma, 2009a
10	Jungle rice / Awnless barnyard grass	*Echinochloa colona*	Poaceae	Karthikeyan and Sosamma, 2009a
11	Not known	*Arundinella metzii* Hochst	Poaceae	Dalvi *et al*., 1985
12	Kodo millet	*Paspalum scrobiculatum* Linn	Poaceae	Dalvi *et al*., 1985
13	Not known	*Ischaemum travancorence*	Poaceae	Dalvi *et al*., 1985
14	Vetiver bunchgrass	*Vetiver zizanoides* Linn.	Poaceae	Dalvi *et al*., 1985
15	Elephant grass	*Pennisetum purpureotyphoides*	Poaceae	Dalvi *et al*., 1985
16	Not known	*Arundinella* sp	Poaceae	Dalvi *et al*., 1985
17	Guinea grass	*Panicum maximum* Jacq	Poaceae	Dalvi *et al*., 1985
18	Angleton blue stem grass	*Dichanthiumi aristratum* (Poir)	Poaceae	Dalvi *et al*., 1985
19	Para grass/ buffalo grass	*Brachiaria mutica* (Forst)	Poaceae	Dalvi *et al*., 1985
20	Sugarcane	*Sacchrum officinarum* Linn.	Poaceae	Dalvi *et al*., 1985

yield (-0.904) and straw yield (-0.969). Whereas there was a positive correlation between beetle population with leaf damage (+0.991) (Krishna *et al*., 2016).

5.1 Alternate hosts of rice blue beetle, *L. Pygmaea*

The *L. pygmaea* feeds/survive on more than twenty alternate hosts during offseason belongs to diversified families of Cyperceae, Limnocharitaceae, Lythraceae, Pontederiaceae and Poaceae as mentioned in Table 1.

6. Integrated pest management strategies

Management strategies mainly consist of mechanical and cultural methods by planting of cultivars that have genetic resistance to insects, biological agents (parasites, predators and diseases) and use of insecticides. Income per hectare in rice production is relatively low and money spent for controls such as insecticide significantly lesser profits. Insecticide prices are increasing faster than the rice price in most countries including India.

6.1 Mechanical and Cultural Methods

There are several mechanical and cultural methods recommended to control *L. pygmaea* pest earlier by spreading kerosene in the standing water in rice fields and dislodging the stages of pest by means of local devices to control the blue beetle (Usman, 1947). Plucking the infected leaves if lesser incidences and uprooting of the whole plant in case of severe incidence. The closer spacing (10 × 10 cm and 10 × 15 cm) in rice has significantly reduced the damage incidence by 59.02% as compared to 20 × 15 cm spacing in Kerala (Karthikeyan and Sosamma, 2008b). The early transplanting of rice during July 1st week has significantly reduced the *L. pygmaea* beetle incidence (46.47%) and damage (57.71%) compared to delay transplanting during last week of August in Karnataka (Krishna *et al*., 2013a).

6.2 Biological control

Spiders, amongst the predators, are the most familiar and ubiquitous obligatory carnivores, which feed on different types of prey. In the past, multiple researchers have documented the predatory capabilities of spiders in controlling rice pests both in India and other countries. (Nath and Sarkar, 1978; Kiritani, 1979; Ghode *et al*., 1985). In rice ecosystem natural enemies play an important role in keeping the pest population below ETL. Spiders and mirid bugs are important predators active throughout crop season (Manjunath *et al*., 1978). A survey conducted on the spider fauna in 13 districts of Karnataka unveiled the presence of 45 genera belonging to 15 families. (Ansari and Pawar, 1992). Monitoring of the incidence of spiders in rice ecosystem revealed three peaks in the population of spiders *i.e.*, April-May, July and early September.

The abundance of spiders was more in monocropping compared to mixed cropping, but the species diversity was opposite of abundance. It showed that a female spider of *T. mandibulata* consumed on an average 1.02 full grown larvae of rice blue beetle, *L. pygmaea* per day (Patel *et al*., 2013).

The endoparasitoids on *L. pygmaea* were reported are *Chrysonotomyia* sp. (Eulophidae) and *Trichomalopsis* sp. (Pteromalidae) Chalcid wasps belongs to the superfamily Chalcidoidea and order Hymenoptera.

6.3 Chemical Control

Throughout Asia, insecticides are more important component in the control of blue beetle, especially where resistant varieties are not available. However, several factors complicate the use of insecticides in the control of this pest. The rice blue beetle can be managed by spraying of Profenophos 50 EC @ 2 ml/l, Chlropyriphos @ 2.5 ml/litre or Quinalphos @ 2.5 ml/litre which found very effective in reducing its population. The other alternatives to these chemical insecticides are botanicals, *Vitex negundo* aqueous leaf extract @ 5% and entomopathogenic fungi, *Beauveria bassiana* @ 2 g/l (Krishna *et al*., 2012; Karthikeyan and Sosamma, 2010).

7. Current research and future directions

Researchers have been studying the biology and behaviour of *L. pygmaea* to gain a better understanding of its life cycle, feeding habits, reproduction, and host plant preferences. These studies help in identifying potential control strategies and management practices. There has been an increasing focus on developing sustainable and environmentally friendly pest management approaches. These studies contribute to a better understanding of the beetle's evolutionary history, population dynamics, and potential mechanisms underlying adaptation to different environments. To stay updated with the latest developments in *L. pygmaea* research, it is recommended to refer to scientific journals, conferences, and publications.

Investigating the impact of these interactions on the beetle's behaviour, dispersal, and reproductive success would provide important insights for developing integrated pest management strategies. Investigating the chemical ecology of *Leptispa pygmaea* and identifying the specific pheromones involved in mate attraction, aggregation, and host selection could provide opportunities for developing effective monitoring and control strategies. Additionally, understanding the variation in pheromone production among different beetle populations and its implications for population dynamics would be beneficial. Understanding the genetic basis of resistance and exploring alternative control methods, such as biopesticides or host plant resistance, would be essential for sustainable pest management.

8. Conclusion

Rice blue beetle, *Leptispa pygmaea* Baly earlier considered as minor pest has started cause outbreaks which is much concern in rice cultivation regions of India and other countries. Weather factors, with a dominant role on the survivability, development and reproductive capacity of insect pests exert a great influence on their population dynamics.

Through this comprehensive overview, we aim to consolidate existing knowledge on *Leptispa pygmaea* Baly and its impact on rice production. By understanding its biology, distribution, feeding habits, and economic implications, researchers and policymakers can develop effective management strategies tailored to the specific challenges posed by this emerging pest.

The review underscores the need for integrated pest management approaches and encourages further research to minimize the damage caused by *L. pygmaea* and ensure the sustainability of rice production in the face of this growing threat.

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Harnessing the power of drought-resistant microbial inoculants for sustainable agricultural practices

Shilpa M E
Assistant Professor, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga

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ABSTRACT

The increasing frequency and intensity of droughts due to climate change are posing significant challenges to global agriculture. In response, drought-resistant microbial inoculants have emerged as a promising solution to enhance crop resilience under water-stressed conditions. These microbial formulations, including bacteria, fungi, and algae, work synergistically with plants to improve water-use efficiency, boost nutrient uptake, and stimulate stress-responsive mechanisms. By promoting stronger root development, improving soil structure, and enhancing plant water retention, microbial inoculants offer a sustainable alternative to conventional drought management strategies. This article explores the mechanisms by which microbial inoculants enhance drought tolerance in crops, their benefits for soil health and nutrient cycling, and their potential to reduce reliance on chemical fertilizers and irrigation. The adoption of microbial inoculants presents a viable pathway for promoting sustainable agricultural practices and ensuring food security in the face of climate-induced water scarcity.

KEY WORDS: *Drought-resistant microbes*; *Microbial inoculants*; *Sustainable agriculture*; *Crop resilience*

1. Introduction

Agriculture, the backbone of global food security, is increasingly threatened by the changing climate, particularly prolonged droughts. With unpredictable weather patterns and insufficient water resources, farmers around the world are experiencing severe challenges in ensuring consistent crop yields. Conventional irrigation methods are often not sustainable in the face of growing water scarcity, and reliance on chemical fertilizers and pesticides is raising environmental concerns (Vurukonda *et al*., 2016).

To address these challenges, a revolutionary approach has emerged: the use of drought-resistant microbial inoculants. These beneficial microorganisms, when introduced to soil or plant systems, have the potential to significantly improve drought tolerance, enhance soil health, and promote sustainable agricultural practices (Bacchus and Muir, 2020; Singh and Shukla, 2019).

In this article, we will explore the role of drought-resistant microbial inoculants in modern agriculture, their benefits, and mechanisms of action, and potential for widespread adoption across diverse agricultural systems.

CONTACT Shilpa M E meshilpa99@gmail.com

2. Understanding Drought-Resistant Microbial Inoculants

Drought-resistant microbial inoculants are a class of naturally occurring or genetically modified microorganisms - such as bacteria, fungi, and algae - that are capable of enhancing a plant's tolerance to water stress (Liu and Liu, 2021). These inoculants are typically introduced to the soil or applied directly to plant roots, where they interact with plants in various beneficial ways. Their primary role is to mitigate the harmful effects of drought on crops by improving the plant's water-use efficiency, enhancing nutrient uptake, and promoting soil health (Brimecombe and He, 2017).

The term "microbial inoculants" refers to the deliberate introduction of specific microorganisms to the soil or plant environment to improve agricultural productivity. In the case of drought resistance, these microorganisms help crops survive during periods of insufficient water by altering soil conditions, improving water retention, and stimulating plant defence mechanisms (Masi and Sanna, 2020). For instance, some drought-resistant microbes produce substances that retain moisture in the soil or enhance the ability of plant roots to access deeper layers of water. Others may trigger physiological changes in plants, such as the production of stress-related hormones, which enable crops to cope better with environmental stress (Subramanian and Parameswaran, 2020).

3. Mechanisms of Action of Drought-Resistant Microbial Inoculants

The effectiveness of drought-resistant microbial inoculants lies in their diverse mechanisms of action, which can vary depending on the type of microorganism and the crop species. Broadly speaking, these mechanisms can be classified into the following categories:

Water retention and soil structure enhancement

Certain drought-resistant microbes, such as beneficial fungi and bacteria, help to improve the soil's water-holding capacity. Mycorrhizal fungi, for instance, form symbiotic relationships with plant roots and extend their hyphal networks deep into the soil, allowing plants to access water from deeper soil layers that they would not be able to reach otherwise. Furthermore, these fungi can help improve soil structure by aggregating soil particles, increasing water retention and preventing water runoff (Subramanian & Parameswaran, 2020).

Improved root development

Many microbes, including nitrogen-fixing bacteria and plant-growth-promoting rhizobacteria (PGPR), stimulate the growth of plant roots. Stronger, deeper root systems allow crops to access water and nutrients more effectively, especially during dry conditions. This enhanced root architecture ensures that plants are better equipped to survive prolonged drought spells.

Enhanced nutrient uptake

Drought stress can often lead to a reduction in the availability of essential nutrients like nitrogen, phosphorus, and potassium. Certain microbes can help mitigate this issue by enhancing nutrient uptake from the soil. Nitrogen-fixing bacteria, for example, convert atmospheric nitrogen into a form that plants can absorb, reducing the need for synthetic fertilizers. Other microbes secrete

enzymes or organic acids that break down soil-bound nutrients, making them more bio-available to plants.

Production of osmotic regulators and stress-responsive compounds

Some drought-resistant microbes' help plants adapt to water stress by producing osmotic regulators, such as exopolysaccharides, that help maintain cell turgor and prevent wilting. In addition, certain microbes stimulate the production of plant hormones, such as abscisic acid, which regulate the plant's response to drought stress. This can lead to improved stomatal regulation and a better ability to conserve water during periods of drought.

Induced Systemic Resistance (ISR)

Beyond direct effects on water and nutrient availability, drought-resistant microbial inoculants can also activate the plant's internal defence mechanisms. These microorganisms can trigger the plant's immune system, inducing a state of systemic resistance that prepares the plant to better handle environmental stresses, including drought. This phenomenon is particularly beneficial because it enhances the plant's resilience without the need for additional external inputs like fertilizers or pesticides (García and Buján, 2018).

4. Benefits of Using Drought-Resistant Microbial Inoculants in Agriculture

The application of drought-resistant microbial inoculants offers numerous benefits to farmers, the environment, and the broader agricultural system. Some of the key advantages include:

Enhanced drought tolerance and yield stability

One of the most significant advantages of using drought-resistant microbial inoculants is their ability to increase crop tolerance to drought stress (Bacchus and Muir, 2020). By improving water-use efficiency, these inoculants help crops maintain growth and yield during periods of insufficient rainfall or irrigation. This stability in crop production is critical for ensuring food security in regions that are vulnerable to climate change and erratic weather patterns.

Reduction in water usage

Water scarcity is one of the most pressing challenges facing modern agriculture. Microbial inoculants can play a key role in reducing the amount of water required for crop production. By improving the plant's ability to access and conserve water, these inoculants reduce the dependency on irrigation, which can help conserve water resources in regions where water availability is limited.

Improved soil health

Microbial inoculants are not only beneficial to plants but also to the soil ecosystem. They enhance soil fertility, increase microbial diversity, and promote the build-up of organic matter. Over time, this results in healthier soils that are more resilient to drought and other environmental stressors. Healthy soils are essential for maintaining long-term agricultural productivity, particularly in the face of changing climate conditions.

Reduction in chemical inputs

The use of microbial inoculants can reduce the need for chemical fertilizers and pesticides, which are often harmful to the environment and human health. By enhancing nutrient uptake and promoting plant health, microbial inoculants reduce the reliance on synthetic chemicals, leading to more sustainable and eco-friendly farming practices.

Cost-effectiveness

In many cases, microbial inoculants are cost-effective compared to traditional drought management techniques, such as the installation of irrigation systems or the purchase of expensive chemical fertilizers. While the initial investment in microbial inoculants may vary, their long-term benefits - such as reduced water usage, improved yields, and lower input costs - make them an attractive option for many farmers.

5. Challenges and Limitations

While the potential benefits of drought-resistant microbial inoculants are significant, there are several challenges and limitations that need to be addressed for their widespread adoption.

Variable efficacy

The effectiveness of microbial inoculants can vary depending on a number of factors, including soil type, climate conditions, and crop species. Not all microbes are suited to all environments, and their performance can differ from one location to another. Additionally, some microbial inoculants may only provide benefits under certain conditions, making it essential for farmers to choose the right product for their specific needs.

Regulatory and commercial challenges

The commercialization of microbial inoculants faces regulatory hurdles in many regions. There is a need for standardized testing and certification to ensure that these products are safe and effective. Furthermore, the microbial inoculant market is still developing, and the availability of high-quality, region-specific products may be limited in certain areas.

Knowledge gaps and training needs

For many farmers, the concept of using microbial inoculants may be unfamiliar, and there is a need for education and training on their use and benefits. Extension services, research institutions, and agricultural organizations will need to play a key role in disseminating knowledge and supporting farmers in adopting these technologies.

Cost of initial investment

While microbial inoculants can be cost-effective in the long run, the initial investment may be prohibitive for some small-scale farmers. Access to affordable inoculants and appropriate financing options will be crucial in making these technologies accessible to farmers across different economic strata.

6. Future Prospects and Conclusion

The use of drought-resistant microbial inoculants represents a promising strategy for enhancing crop resilience in the face of climate change and water scarcity (Liu and Liu, 2021). As research continues to advance, we can expect to see new strains of microorganisms that are specifically tailored to different environmental conditions and

crops. The integration of microbial inoculants into sustainable farming practices has the potential to revolutionize agriculture, providing farmers with an effective, eco-friendly tool to manage drought stress and improve yields.

However, realizing the full potential of these technologies will require collaboration among researchers, policymakers, agricultural companies, and farmers. By overcoming the challenges associated with microbial inoculants, we can move toward a more sustainable and resilient agricultural future, ensuring that food production can meet the demands of a growing global population, even in the face of climate uncertainty.

In conclusion, drought-resistant microbial inoculants offer a powerful and innovative solution to one of the most pressing challenges facing agriculture today. With the potential to enhance drought tolerance, improve soil health, reduce chemical inputs, and promote sustainable farming, these microbial technologies could play a key role in ensuring global food security in the face of climate change. As we move toward a more sustainable agricultural future, microbial inoculants may become an indispensable tool for farmers around the world, helping to safeguard crops, livelihoods, and ecosystems for generations to come.

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