

Study on Genetic Variability, Genetic Divergence and Identification of Zinc Rich and High Yielding Genotypes in Cowpea [*Vigna unguiculata* (L.) Walp.] Germplasm

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ABSTRACT

The present experiment was to assess genetic variability in 263 cowpea germplasm accessions for yield and its related traits and identify high yielding genotypes rich in zinc and iron. Analysis of variance for yield and its related traits recorded significant difference among the genotypes, indicating there is sufficient variability among the genotypes used in the study. Genetic variability estimates were high for seed yield per plant, moderate for clusters plant⁻¹, pods plant⁻¹, pods cluster⁻¹ and 100-seed weight. High heritability coupled with high genetic advance as per cent of mean was observed for the above-mentioned traits except for pods plant⁻¹ and pods cluster⁻¹ showed moderate h² and GAM, indicating the influence of additive genes in controlling these traits. Cowpea germplasm were grouped into 3 clusters following K-mean clustering with maximum number of 167 accessions in cluster II. Germplasm accessions EC100087 (94.1 ppm), IC426801 (86.5 ppm) of Cluster I and EC18118 (68.6 ppm) of Cluster II were identified as genetically divergent with zinc rich and high seed yield plant⁻¹ and these accessions shall be used in cowpea biofortification programmes.

Keywords : Cowpea, Variability, Genetic divergence, K- mean clustering, Zinc content

COWPEA [*Vigna unguiculata* (L.) Walp.] is an important leguminous crop belonging to family *Fabaceae*. Ploidy level is diploid with chromosome number $2n = 22$. Like any other pulses, cowpea is rich source of protein, fibre, vitamins and minerals. It is an essential crop extensively grown in the *kharif* and *summer* seasons in most parts of India. Cowpea is believed to be used as a food source ever since Neolithic times. It has originated in Africa and is recognized as a pulse crop of tropical and subtropical regions.

It is a tropical grain legume that plays an important nutritional role in developing countries by contributing to food security. Cowpea grains contain about 64 per cent carbohydrate and 23-28 per cent

protein. Due to the presence of high protein content, it is generally referred to as 'Poor man's meat' and therefore, has greater potential in alleviating malnutrition (Bressani 1985; Singh *et al.*, 1997 and Lal & Vashisht, 2008). Cowpea is also an excellent source of amino acids and micronutrients *viz.*, iron and zinc. Dry seeds of cowpea are used to prepare several snacks and main meal dishes. Besides being used in the form of seed, cowpea immature pods are utilized as vegetable and also it is excellent source of green and also dry fodder. It contains a high amount of carbohydrate (60.3%), fat (1.8%) and sufficient amount of calcium (76mg/100 g.), iron (57mg/100g) and vitamins such as thiamine (0.92mg/100g), riboflavin (0.18mg/100 g) and nicotinic acid (1.9mg/100g) (Chatterjee and Bhattacharya, 1986). It is indeed

a great source of essential fatty acids, such as linoleic and linolenic acids (Augustin and Klein, 1989).

Iron and zinc are the essential micronutrients that catalyze human metabolism and their deficiency causes malnutrition and hidden hunger. This deficiency has negative impact on human health and affects more than two billion people all over the world (Food and Agriculture Organization of United Nations, 2019; Lopez, 2020 and Silva, 2021). Zinc is an important mineral which increases immunity against diseases and its deficiency leads to several health problems (Ayeni *et al.*, 2018 and Van, 2020).

Recent studies have revealed that the zinc concentration in cowpea is much higher than common bean grains (Coelho, 2021 and Gerrano, 2019) and that there is a great genetic variability for the protein, iron and zinc content in the cowpea germplasm grain (Carvalho, 2012; Dias, 2021; Santos & Boiteux, 2015 and Weng, 2019). Therefore, examination of iron and zinc contents in the grains of cowpea genotypes provide information that aids in selection and development of biofortified cultivars for these micro minerals (Dias, 2021).

Improving yield comparably the prime objective of cowpea breeding programmes so far. Along with improving yield there is a need of overcoming micronutrient malnutrition through biofortification. Even though several biofortification strategies could be followed, development biofortified cowpea through conventional breeding approaches provides an eco-friendly and sustainable solution. pushpa *et al.* (2023) reported the effect of foliar application of nutrients as lead to increasing productivity. The success of development of cowpea biofortification mainly depends upon the genetic variability present in the population and the heritability of desirable traits. Extend and type of the genetic variability help the breeder to understand the criteria to practice selection and breeding schemes to be followed. A quantitative assessment of genetic divergence among cowpea germplasm and contribution of various traits towards the divergence helps the breeder by providing information to take up hybridization programme and

genetic improvement of yield (Nagalakshmi *et al.* 2010). Present investigation aims in studying genetic variability present in cowpea germplasms, genetic divergence between them and identifying superior genotypes rich in zinc content and yield attributing traits.

MATERIAL AND METHODS

The experimental material consisted of 263 cowpea (*Vigna unguiculata* L. Walp) germplasm accessions including five checks namely PL-3, PL-4, C-152, KBC-9 and KBC-2. The germplasms were collected from NBPGR, New Delhi, University of Agricultural Sciences, Shimogga, College of Agriculture, V.C. Farm, Mandya and G.B. Pant University of Agriculture, Panthnagar. Experiment was carried out during *summer* 2021 at experimental fields of K-Block, GKVK, UAS, Bangalore. Geographically, the experimental site is located at 13° 00'N latitude and 77° 35'E longitude. Augmented experimental design (Federer, 1956) was used with seven blocks, each block with 45 germplasm and a total of 263 germplasm. The germplasms were unreplicated, while checks were replicated twice in all the blocks to obtain the estimate of an error and blocking effects. Individual genotypes were directly sown by dibbling one seed per hill with 30 cm spacing between plant to plant and 45 cm space between row to row. The field management was done by following the recommended packages of practices. Fifty per cent of the total nitrogen was applied at 30 DAS while remaining 50 per cent of nitrogen whole DAP and MOP were applied as basal dose. Observations *viz.*, days to 50 per cent flowering, clusters plant⁻¹, pods cluster⁻¹, pods plant⁻¹, pod length (cm), hundred seed weight (g) and seed yield plant⁻¹ (g) were recorded on randomly selected five plants in each germplasm.

Statistical Analysis

The genotypic and phenotypic coefficient of variation was computed according to Burton and Devane (1953). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were classified as suggested by Sivasubramanian and Menon (1973) that are, low (<10%), moderate (10-20%) and high (>20%). The heritability

percentage was classified as low (0-30%), moderate (30-60%) and high (>60%) by Robinson *et al.* (1949). Genetic advance was computed by using the formula given by Robinson *et al.* (1949) and genetic advance over mean was calculated by given formula. The genetic advance as *per cent* mean was categorized as low up to 10 per cent, 10 to 20 per cent consider as a moderate and more than 20 per cent noticed as a high (Johnson *et al.*, 1955).

K - Mean Clustering

Classification of germplasm accessions were done following model-base K-means clustering approach (Mac queen, 1967) to unravel organization of variability using R software *ver.* 4.2.1. F test and Levene's test (1960) were used for testing their homogeneity across the clusters and trait means and variances were estimated.

Analysis of Zinc Content (mg kg⁻¹)

Estimation of Zinc in dry seeds in each generation was done using Atomic Absorption Spectrophotometer (AAS) (Jackson *et al.*, 1973). The protocol is briefly described below.

- Exact 0.5g of dried seed sample was taken and powdered
- 10ml of tri-acid containing HNO₃, H₂SO₄ and Perchloric acid in the ratio of 9:2:1 v/v was added to sample in conical flask
- The sample was digested under heater at 120°C in a fume-hud until the volatile content disappears retaining only inorganic minerals for about 2 to 2.5 hrs.
- Carefully the flask was taken out of the heat and cooled to room temperature
- The extracted mineral volume was made up to 100ml in volumetric flask and sample was flittered with wahtman's number 1 filter paper
- The extract was stored until taken as aliquot part for mineral estimation
- Samples are fed to atomic absorption spectrophotometer (AAS) and values are recorded.

Concentration of ppm is calculated using formula :

$$\text{Zn (ppm)} = \frac{\text{Graph ppm} \times \text{volume digested}}{\text{Weight of samples}}$$

RESULTS AND DISCUSSION

Genetic Variability

Genetic variability is prerequisite to practice selection in breeding of any crop and so as in cowpea. ANOVA results confirmed the significant variations present for each and every trait under the study among the germplasm and also for the checks used for study. Block effects were significant for clusters plant⁻¹ and pods cluster⁻¹ suggesting the prevalence of variability because of heterogeneous area. The detailed information is provided in Table 1. Mean performance of germplasms for seven yield traits is presented in Table 2.

PCV estimate was high for seed yield plant⁻¹ (23.59%), moderate for pods plant⁻¹ (19.44%), pods cluster⁻¹ (17.88%), clusters plant⁻¹ (16.67%) and hundred seed weight (10.54%). Similarly, Khandait *et al.* (2016) reported high PCV and GCV values for number of pods cluster⁻¹. Low estimates of PCV was recorded for days to 50 per cent flowering (8.60%) and pod length (7.54%), while high GCV for seed yield plant⁻¹ (20.35%). Our results were in accordance with Arriyo (1995) who evaluated 20 cowpea varieties and high PCV of 35.25 per cent and GCV of 22.11 per cent for seed yield plant⁻¹. Lohithaswa *et al.* (2009), Chattopadhyay *et al.* (2014), Khanpara *et al.* (2015), Chandrakar *et al.* (2016), Ngoc *et al.* (2019) and Vinay *et al.* (2022), also recorded high PCV and GCV estimates for seed yield plant⁻¹ in their studies conducted on cowpea genotypes. Moderate GCV was noticed for pods cluster⁻¹ (12.76%), pods plant⁻¹ (16.03%), clusters plant⁻¹ (13.44%) and 100-seed weight (10.30%). Similarly, Lesly (2005) where they evaluated 169 genotypes and recorded high PCV and GCV values for seed yield plant⁻¹, hundred seed weight and number of pods plant⁻¹. Omoigui *et al.* (2006) also observed high GCV for hundred seed weight. Contrasting results were observed by Vidya

TABLE 1
Mean sum of squares for yield and its attributing characters in 263 germplasm lines

Source	Degrees of freedom	Days to 50 per cent flowering	Clusters plant ⁻¹	Pods cluster ⁻¹	Pods plant ⁻¹	Pod length (cm)	Hundred seed weight (g)	Seed yield plant ⁻¹ (g)
Blocks	6	2.01	7.35 **	0.38 **	8.80	2.28	0.04	3.41
Germplasm + Checks	267	9.68 **	46.30 **	0.51 **	155.21 **	7.39 **	6.39 **	337.82 **
Germplasm	262	9.71 **	20.70 **	0.49 **	96.04 **	7.11 **	6.19 **	296.55 **
Checks	4	7.09 *	264.55 **	1.95 **	168.36 **	11.87 **	9.15 **	1173.83 **
Germplasm vs Checks	1	12.41 *	5878.03 **	0.06 **	15479.00 **	61.52 **	45.95 **	7752.25 **
Error	59	2.36	1.48	0.06	6.05	0.58	0.06	6.83
S.E.D		1.88	1.40	0.28	2.82	0.87	0.29	3.00
CD@0.5		3.76	2.70	0.55	5.64	1.74	0.58	5.99

Note : *Significant at P=0.05; **Significant at P=0.01; S.E.D= Standard error of difference means; CD= Critical difference

TABLE 2
Descriptive statistics for seven quantitative characters in 263 cowpea germplasm lines

Characters	Mean ± SE	Range		Absolute Range	Standardized Range
		Minimum	Maximum		
Days to 50 per cent flowering	43.06 ± 0.24	36.00	56.00	20.00	0.46
Clusters plant ⁻¹	12.33 ± 0.33	3.20	24.00	20.80	1.68
Pods cluster ⁻¹	1.94 ± 0.04	1.00	4.00	3.00	1.54
Pods plant ⁻¹	22.37 ± 0.64	4.00	68.80	64.80	2.89
Pod length (cm)	16.29 ± 0.17	8.60	23.80	15.20	0.93
Hundred Seed weight (g)	11.06 ± 0.15	6.40	20.00	13.60	1.13
Seed yield plant ⁻¹ (g)	33.06 ± 1.08	9.50	114.21	104.80	3.16

SE = Standard error

and Oommen (2002) for traits such as pods plant⁻¹, number of pods plant⁻¹ and pod weight in 50 varieties of cowpea where they recorded high GCV for pods plant⁻¹. The present study suggested that there is considerable amount of variation in the characters under study and can be improved through practicing selection. The estimates of GCV were low for days to 50 per cent flowering (7.87%) and pod length (5.93%). Similarly, Ajmera *et al.* (2017) reported low estimates of PCV and GCV for pod length but high PCV and GCV estimates for days to 50 per cent flowering. Contrasting results was observed by Nwofia *et al.* (2012), Chattopadhyay *et al.* (2014), Santos *et al.*

(2014) and Khanpara *et al.* (2015) for pod length. Narrow difference between PCV and GCV for all the characters indicates low influence of environment and phenotype truly represents the genotype. The results were similar with the findings of Manisha *et al.* (2018), Bamji & Pithia (2020), Ugale *et al.* (2020) and Tatis *et al.* (2021).

Heritability was high for traits such as hundred seed weight (95.58%) followed by seed yield plant⁻¹ (86.15%), days to 50 per cent flowering (83.70%), pods plant⁻¹ (68.00%), clusters per plant (64.96%) and pod length (61.800%) indicating that selection for these

characters will be effective since they are least influenced by the environment. Heritability was moderate for pods cluster⁻¹ (50.95%). Khanpara *et al.* (2015) observed high heritability for pod yield plant⁻¹, pod length, seeds per plant, pods per cluster and 100-seed weight. Our results were similar with the findings of Devi & Jayamani (2018), Nair *et al.* (2018), Singh *et al.* (2018), Nkoana *et al.* (2019) and Tambitkar *et al.* (2021). Johnson *et al.* (1955) reported that heritability estimates along with genetic gain will be more effective than the former alone in predicting the effectiveness of selection. Therefore, it is essential to consider predicted genetic advance along with heritability estimate to increase the efficiency of selection. A relative comparison of heritability values and expected genetic gain estimated as per cent of mean (GAM) will provide knowledge about the nature of gene action governing for a particular character.

Estimates of high heritability coupled with high GAM were observed for seed yield plant⁻¹ followed by hundred seed weight, pods plant⁻¹ and clusters plant⁻¹. These characters are governed by additive-gene action and possess high selective value and thus selection pressure could profitably be applied to these characters for their improvement. Present results were similar to the studies of Rout *et al.* (2018), Swathi *et al.* (2019), Gupta *et al.* (2019) and poornim *et al.* (2023). High heritability coupled with moderate GAM were observed for days to 50 per cent flowering and

moderate heritability and GAM were recorded for pods per cluster. Results show the governance of both additive and non-additive gene action for the characters. Our results were in accordance with the findings of Lal *et al.* (2017) and Belay & Fisseha (2021) where they observed high heritability and moderate genetic advance for pod yield plant⁻¹, pods plant⁻¹, pod length and pods plant⁻¹. High heritability coupled with low GAM were recorded for pod length this may be due to the presence of non-additive gene action. Similar results were recorded by Surpura *et al.* (2017) and Rai *et al.* (2020). Our results suggest that the improvement of these characters can be done through selection and through combination breeding (Table 3). Variability with respect to pod color and shape is presented in Plate 1.

K – Mean Clustering

In plant breeding accessions contrasting for economically important traits is a prerequisite. Cluster analysis groups the accessions with similar characters into different clusters and aids in identification of genetically diverse and desirable genotypes. In our study, out of 263 germplasm, 167 germplasm lines were grouped under cluster II, 57 germplasm lines were grouped under cluster III and cluster I had least germplasm lines of 38 with K-value ranging from 2.3 to 5.1 (Fig. 1). Significant traits mean differences between three clusters was recorded for all the

TABLE 3

Variability, heritability and genetic advance for yield and its attributes in 263 cowpea germplasm lines

Characters	Mean	PCV (%)	GCV (%)	$h^2_{(bs)}$ (%)	GAM (%)
Days to 50 per cent flowering	43.06	8.60	7.87	83.70	14.85
Clusters plant ⁻¹	12.33	16.67	13.44	64.96	22.22
Pods cluster ⁻¹	1.94	17.88	12.76	50.95	18.35
Pods plant ⁻¹	22.37	19.44	16.03	68.00	27.13
Pod length (cm)	16.29	7.54	5.93	61.80	9.51
Hundred seed weight (g)	11.06	10.54	10.30	95.58	29.74
Seed yield plant ⁻¹ (g)	33.06	23.59	20.35	86.15	41.86

GCV = Genotypic co-efficient of variation, PCV = Phenotypic co-efficient of variation, $h^2_{(bs)}$ = Heritability Broad sense, GAM = Genetic advance as per cent of mean



Plate 1 : Variability for seed colour among selected germplasm with high zinc content

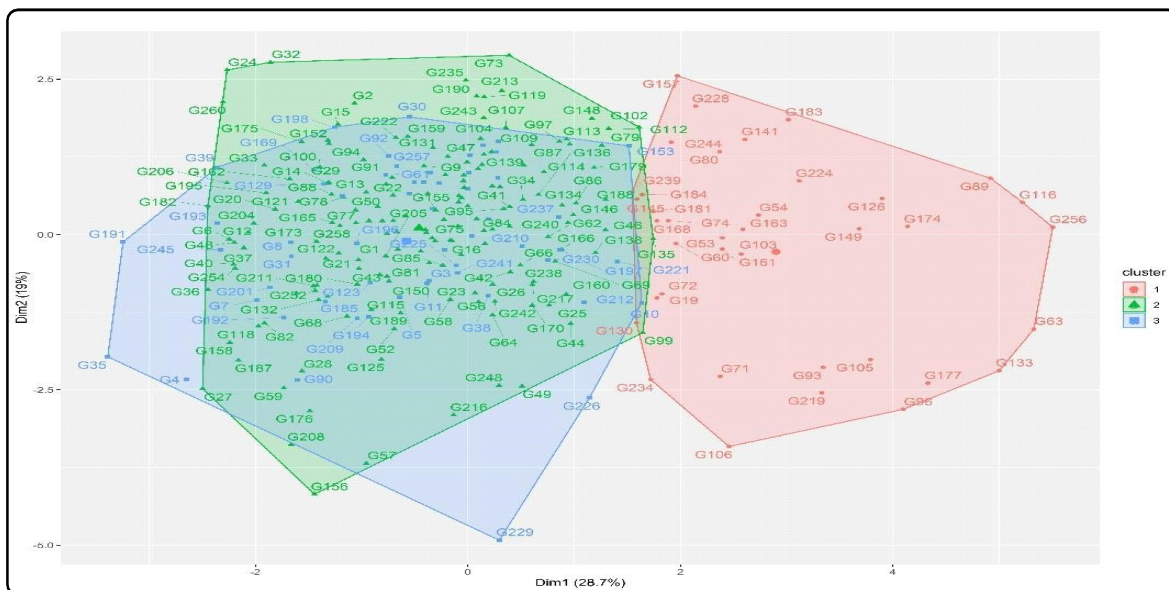


Fig. 1 : Graphical representation of 263 cowpea germplasm into three different cluster formed based on K-means clustering

characters under consideration except pods per cluster (Table 4). The trait variances among three clusters were significant for six out of the seven quantitative traits such as days to 50 per cent flowering, clusters plant⁻¹, pods plant⁻¹, pod length, 100-seed weight and seed yield plant⁻¹ (Table 4). Between the three clusters, cluster I recorded higher mean values for days to 50 per cent flowering (45.0 days), cluster per plant (16.6), pods per cluster (2.3), pods per plant (35.9), pod length (17.1 cm) and 100-seed weight (15.3g). Cluster II recorded lowest mean values among the three clusters for traits such as days to 50 per cent

flowering (43.9 days), cluster per plant (6.8), pods per cluster (1.8), pods per plant (15.8), pod length (16.3cm) and 100-seed weight (10.6g). Overlapping of the two clusters indicate similarity of mean between them. Concurrent results were reported by Saini *et al.* (2004), where 155 cowpea lines were grouped into ten clusters and cluster I had maximum number of germplasm lines. Out of the ten clusters, Cluster IX recorded highest mean values for seed yield per plant and 100 seed weight. Similarly, several researchers such as Jain *et al.* (2006), Bhandari & Vema (2007), Dalsaniya *et al.* (2009), Ahamed *et al.* (2014) and

TABLE 4
Mean comparison profile of cowpea germplasm lines into different clusters by K-mean clustering method

Characters	C1	C2	C3	F value	P value
Days to 50 per cent flowering	45.05	43.91	44.26	1.82 *	0.03
Clusters plant ⁻¹	15.62	9.40	10.95	2.01 *	0.01
Pods cluster ⁻¹	2.39	1.84	1.98	1.114	0.46
Pods plant ⁻¹	35.94	15.83	19.66	11.75 *	0.036
Pod length (cm)	17.17	16.39	16.84	3.60 *	0.001
100-seed weight (g)	12.50	10.62	11.21	56.48 *	0.003
Seed yield plant ⁻¹ (g)	64.36	23.77	32.10	11.15 *	0.003

C = Cluster

Brahmaiah *et al.* (2014) evaluated cowpea genotypes, assessed genetic divergence and obtained different number of clusters with significant difference between each cluster. Results indicated that K-mean clustering approach is efficient in minimizing within cluster variance and maximise between-cluster variance by grouping diverse accessions into different clusters. The estimates of means of quantitative traits such as days to 50 per cent flowering, clusters plant⁻¹, pods cluster⁻¹, pods plant⁻¹, pod length, 100-seed weight and seed yield plant⁻¹ were highest among the accessions included in cluster I and were least among the accessions included in cluster II. Therefore, it is desirable to choose germplasm accessions from among those included in Cluster I and cluster II in cowpea breeding programme to recover maximum number of superior transgressive segregants with combination of desirable traits. In accordance with our study, Borah and Fazlullah Khan (2001) studied 60 fodder cowpea genotypes and grouped them into ten clusters out of which cluster I, II, IX and X were most divergent from other clusters with respect to traits mean values.

Classification of Cowpea Germplasm Based on Seed Zinc Content

Top sixty germplasm were selected from 263 germplasm which outcrossed checks for seed yield per plant and were used for micronutrient estimation. Zinc analysis revealed all the selected genotypes differed significantly among themselves. In the present study, seed zinc content ranged from 94.19 to 1.0 ppm with mean value of 29.62 ppm. The germplasm line EC100087 recorded highest zinc content of 94.20 ppm with seed yield plant⁻¹ of 76.4g followed by germplasm IC426801 and EC18118 with zinc content of 86.5 ppm and 68.6 ppm, respectively and seed yield plant⁻¹ 104.7g and 98.2g, respectively (Table 5). Singh *et al.* (2004) recorded zinc content of 46 ppm in IT97K-1042-3 genotype of cowpea. In our study, germplasm line IC488085 recorded lowest zinc (1.0 ppm) with seed yield plant⁻¹ of 63.5 g among 60 germplasm. The differences between the zinc content and seed yield plant⁻¹ in the selected germplasms might be due to difference in genetic

TABLE 5
Top ten selected cowpea germplasm lines for zinc content

Germplasm	Zinc (ppm)	Seed yield plant ⁻¹ (g)
EC100087	94.20	76.40
IC426801	86.50	104.70
EC18118	68.60	92.20
IC341091	66.50	66.20
IC554350	63.50	75.30
IC282076	54.30	41.50
IC201077	44.40	93.20
IC606360	44.20	64.00
EC240829	41.80	50.90
EC149273	40.20	70.00
KBC-9	34.21	45.15
PL-4	58.05	36.00
KBC-2	32.90	35.95
PL-3	38.00	43.45
C-152	30.00	34.66

makeup of cowpea germplasms to absorb soil zinc and store in the seed. Similarly, Mamiro *et al.* (2011), Santos & Boiteux (2013), Gerrano *et al.* (2015), Marappa *et al.* (2016), Dakora *et al.* (2019) and Gondwe (2019) evaluated cowpea genotypes for zinc content and reported several genotypes rich in zinc content. In the present study, germplasm lines IC426801 (86.5 ppm), EC18118 (68.6 ppm), IC341091 (66.5 ppm) belonging cluster I and EC100087 (94.2 ppm) grouped in cluster II were rich in seed zinc content and recorded higher seed yield plant⁻¹. These lines could be used as parents in future cowpea biofortification programs. In the present study, the accessions tend to have significant variability since they are landraces that have evolved over million years through mutation and recombination (Allard, 1999). These landraces will have several combinations of desirable traits suitable for different environmental conditions. Therefore, usage of these in regular plant breeding programs shall enhance its efficiency in developing cultivars suitable for biotic and abiotic stresses.

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