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ESTIMATES OF GENETIC VARIABILITY OF TOMATO GENOTYPES FOR PHYSIOLOGICAL, NUTRITIONAL AND BIOCHEMICAL PARAMETERS

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ABSTRACT

The present investigation entitled "Estimates of Genetic variability of tomato genotypes for Physiological, nutritional and biochemical parameters" was conducted in randomized block design with 30 genotypes of tomato in three replications. The objectives were to assess the relative performance, estimation of genetic parameters. The characters studied were morphological. The experiment materials comprised of 30 genotypes of tomato were collected from IIVR, Varanasi. The experiment was laid out at Vegetable Research Farm, ICAR- Indian Institute of Vegetable Research, Varanasi. The results of the study revealed that high GCV and PCV estimates were recorded SOD (469.00 and 469.14). The heritability estimates were found to be high (more than 60%) for leaf area index and guaiacol peroxidase (GPX) (100.00). Whereas the genetic advance estimates were found to be high for leaf area index (3299.53). Whereas the genetic advance in % mean estimates were found to be high for SOD (965.86).

Key words : Genetic variability, Physiological, Nutritional, Biochemical, Tomato.

Introduction

Tomato (*Solanum lycopersicum* L. syn. *lycopersicon esculentum* Mill.) belongs to genus *lycopersicum* and the family Solanaceae, also called night shade family and tomato is called in india "poor man's orange" in India whereas called "Love of Apple" in England. Tomato with chromosome number 2n=24. Linnaeus are called *Solanum lycopersicon* and Miller called *Lycopersicon esculentum* (Angadi and Dharmatti, 2012). After Potato and Sweet potato tomato is the World's most important vegetable crop but in India tomato cultivation ranks third after potato and onion. India ranks second in the world of the tomato production and area leading countries after china. The total production of tomato in the world have 1653.40 lakh metric tonnes and Area 47.62 lakh hectare and productivity 42.18 tonnes/ha. In the world tomato India share of area , production and productivity are, 7.8 lakh hectare, 175.78 lakh metric ton and 24.17 tonnes/ha (Anonymous, 2018a). Tomato total demand in India 19.22 million tonnes and India export tomato 47.45 thousand metric ton (Anonymous, 2018b). Tomatoes are high in antioxidants, minerals, and vitamins, eating tomatoes and tomato-based products improves skin health, lowers the risk of cancer and heart disease and lowers bad cholesterol. Tomatoes became extremely popular due to their potential significance. It is often called "Protective Food" due to its high concentration of various nutritive phytochemical compounds, including minerals like phosphorus, calcium, iron and flavonoids, phenolic acids, ascorbic acid and carotenoids like lycopene and â-carotene (Pavankumar *et al.*, 2024). The tomato is a fruit that is essential to human nutrition. It contains 22 calories, 310 I.U. of vitamin A, 1.07 mg of vitamin B1, 94.1% of water, 2.9% of protein, 0.4g of fat, 0.8% of fiber, 3.46% of carbohydrates, 0.71 mg of vitamin B2, 31 mg of vitamin C, 20 mg of calcium, 36 mg of phosphorus, and 0.8 mg of iron. In addition to being valued for their flavor and color, tomatoes are also an excellent source of the antioxidants beta-carotene, ascorbic acid and lycopene (Sinha *et al.*, 2024).

Materials and Methods

The present investigation entitled "Estimates of Genetic variability of tomato genotypes for Physiological, nutritional and biochemical parameters" was conducted in randomized block design with 30 genotypes of tomato in three replications. The objectives were to assess the relative performance, estimation of genetic parameters. The characters studied were morphological. The experiment materials comprised of 30 genotypes of tomato were collected from IIVR, Varanasi. The experiment was laid out at Vegetable Research Farm, ICAR- Indian Institute of Vegetable Research, Varanasi. The experimental site is located at ICAR- Indian Institute of Vegetable Research, Varanasi, about 20 Km south-west of Varanasi situated at 25.18°N latitude and 83.03°E longitude in North Genetic plain in eastern part of Uttar Pradesh (India) and elevation of 128.93 m above mean sea level (MSL). All the parental seedlings were transplanted in the crossing block with spacing of 60 cm × 60 cm on 20th August 2017. The crop was raised as per package of practices of tomato. The mean data of each character was subjected to statistical analysis for variance and test the significance of each character as per the procedure of Panse and Sukhatme (1967). Genotypic and phenotypic coefficients variation were calculated by standard procedures (Johnson *et al.,* 1955 and Hanson *et al.,* 1956). Heritability (h^2 broad sense) and Genetic advance method by Robinson *et al.* (1949) Genetic advance as percentage over mean method by Johnson *et al*. (1955).

Physiological, nutritional and biochemical characters

Chlorophyll-a: The chlorophyll-a was extracted in 80% acetone (Porra *et al*., 1989). The absorption of the extracts at wavelengths of 663 nm and 645 nm for chlorophyll a was recorded with a SP 722E spectrophotometer. The concentrations of chlorophyll a (Chl a) were then calculated using the equations (Arnon, 1949) as follow and concentration was expressed as mg g^{-1} fresh weight.

$$\text{Chl-a} = 12.7A_{663} - 2.59A_{645}$$

Where,

A_{663} = Absorbance at 663 nm wave length

A_{645} = Absorbance at 645 nm wave length

12.7, 2.59 = Absorbance co-efficient

Chlorophyll-b: The chlorophyll-b was extracted in 80% acetone (Porra *et al*., 1989). The absorption of the extracts at wavelengths of 663 nm and 645 nm for chlorophyll b for carotenoid was recorded with a SP 722E spectrophotometer. The concentration of chlorophyll-b (Chl-b) was then calculated using the equations (Arnon, 1949) as follow and concentrations were expressed as mg g^{-1} fresh weight.

$$\text{Chl-b} = 22.9A_{645} - 4.67A_{663}$$

Where,

A_{663} = Absorbance at 663 nm wave length

A_{645} = Absorbance at 645 nm wave length

22.9, 4.67= Absorbance co-efficient

Leaf area index: Portable Leaf area meter used for measuring leaf area index.

Total soluble solid (^{0}Brix): TSS was recorded from blended juice of fruits by using ERMA hand refractometer.

pH: Fruit juice was collected from a single fruit of each genotype by blending it to measure fruit pH using REX pH meter model-PHS-3C. The electrode was inserted into the juice to get pH value.

Beta carotene: Beta carotene was extracted and analysed according to Thimmaih (1999). Briefly, tomato juice (from 5-10 g pulp) was extracted with acetone until the residue is colourless. The acetone extracts were transferred to a separate funnel containing 20 ml petroleum ether and mixed gently. Subsequently, 20 ml of 5% sodium sulphate solvent was added. The two phases formed were separated and the lower aqueous phase was re-extracted with additional petroleum ether, until the aqueous phase was colourless. Petroleum ether extracts were pooled in a brown bottle containing 10 g anhydrous sodium sulphate. After standing, it for ten minutes the petroleum ether extract was decanted in 100 ml volumetric flask through a funnel containing cotton wool. The volume was made up and the absorbance measured using a UV-visible double beam spectrophotometer (Shimadzu-UV-160) at 452 nm using petroleum ether as blank.

Lycopene: Lycopene was extracted and analysed according to Thimmaih (1999). Briefly, tomato juice (from 5-10 g pulp) was extracted with acetone until the residue is colourless. The acetone extracts were transferred to a separate funnel containing 20 ml petroleum ether and mixed gently. Subsequently, 20 ml of 5% sodium sulphate solvent was added. The two phases formed were separated and the lower aqueous phase was re-extracted with additional petroleum ether, until the aqueous phase was colourless. Petroleum ether extracts were pooled in a brown bottle containing 10 g anhydrous sodium sulphate. After standing, it for ten minutes the petroleum ether extract was decanted in 100 ml volumetric flask through

a funnel containing cotton wool. The volume was made up and the absorbance measured using a UV-visible double beam spectrophotometer (Shimadzu-UV-160) at 503 nm using petroleum ether as blank.

Ascorbic acid: Samples of the Riped fruits from the three replications (for parents and hybrids) were analysed for their ascorbic acid content using 2, 6-dichlorphenol indophenol visual titration method (Sadasivam and Manickam, 1992). The red riped fruits were cut into 2-3 mm pieces and 5 g sample was blended with 0.4 per cent oxalic acid. To the 5 ml of supernatant solution, 10 ml acid mixture was added and titrated against the standard dye to a pink end point, which persisted for atleast 15 seconds (V_2). Similar procedure was followed against acid mixture to get blank titre value against standard solution made in 0.4 per cent oxalic acid to get standard titre value (V_1). Ascorbic acid (mg/100g) = Ascorbic acid (mg) content in standard × V_2 × Total sample volume (ml). Materials and Methods : 42 ml of aliquot V_1 Weight of sample.

Acidity: It was analyzed by following standard method given by Sadashivam and Manicam (1992).

Proline: Proline was extracted and estimated according to Bates *et al*. (1973). 100 mg leaves tissues were homogenized in 2 ml of 3% Sulfosalicyclic acid solution using tissue homonizer. The homogenate was centrifuged at 13,000 g for 10 min. 1 ml of the supernatant was the added in to a test tube to which 1 ml of of glacial acetic acid and 1 ml of freshly prepared acid Ninhydrin solution were added. Tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature and then allowed to cool to room temperature and then 2 ml of toluene was added, mixed on a vortex for 20 sec. in a fume hood. The test tube was allowed to stand for at least 10 min allow the separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm in a spectrophotometer. The concentration of proline was standard curve. The concentration of proline was expressed as ug/g fresh weight.

Reagent

(a) Acid Ninhydrin Reagent- For the preparation of acid ninhydrin reagent 0.25 g of ninhydrin was dissolved in a mixture of warm 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid with agigation.

(b) Sulphosalicyclic acid– Sulfosalicyclic acid was prepared by dissolving 3.0 g sulfosalicyclic acid in 100 ml of distilled water.

(c) Glacial acetic acid (99.7 per cent)

(d) Toluene (99.5 per cent)

Super oxide dismutase (SOD): For extraction of superoxide dismutase (SOD), about 200mg fresh leaf sample were homogenized using prechilled mortal and pestle in 5 ml of 100 mM potassium phosphate buffer containing 0.1mM EDTA, 0.1% (V/V) triton × - 100 and 2% (W/V) polyvinyl pyrrolidone (PVP). The extract was filter through muslin cloth and centrifuged at 22000 ×g for 10 min at 4. Supernatant was dialyzed in cellophane membrane tubing's against the cold extraction buffer for -4h. with 3-4 changes of the buffer and then used for assay. The assay mixture in a total volume of 3ml contained 50mM Sodium carbonate-bicarbonate buffer, 0.1mM epinephrine and enzyme. Epinephrine was the last componenet to be added, the adrenchrome formation in the next 4 min was recorded at 475 nm in Uv-v is spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50 % Inhibition of epinephrine oxidation under experimental condition. Activity of SOD was expressed in unit Mg protin.

Assay of guaiacol peroxidase (GPX) activity: Guaiacol peroxidase (EC 1.11.1.7) activity was measured according to the procedure of Shah *et al*. (2001). 200 mg of fresh leaf sample was homogenized in 5 ml of 60 mM sodium phosphate buffer (pH 7.0) in a mortar and pestle pre-chilled at 4ºC. The supernatant, obtained from the centrifugation of homogenates at 22,000 × g for 15 min, was served as enzyme preparation. In a total volume (2 ml) of assay mixture, 40 mM sodium phosphate buffer (pH 6.0), 9 mM guaiacol, 2 mM H_2O_2 and 50 µl enzyme extract were present. At 470 nm (extinction coefficient of 26.6 mM^{-1} cm^{-1}) up to 5 min, the increase in absorbance was recorded and the enzyme-specific activity is expressed as mmol H_2O_2 reduced mg^{-1} (protein) min^{-1}.

Ascorbate peroxides (APX): The ascorbate peroxidase (APX; EC: 1.11.1.11) activity was calculated as suggested by Nakano and Asada (1981). 5 ml of 50 mM potassium phosphate buffer of pH 7.8 that contained 1 mM EDTA, 1 mM ascorbic acid, 1% PVP; and 1 mM phenyl methyl sulphonyl fluoride, 200 mg of fresh leaf sample was blended at 4ºC. For the calculation of enzymes, the supernatant was used after centrifugation at 22,000 × g for 15 min at 4ºC. At 25ºC, the total volume of 3 ml of reaction mixture included 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM AsA, 0.2 mM H_2O_2 and 200 µl enzyme extract. In the reaction mixture, H_2O_2 was mixed at last. By the reduction in absorbance at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}) up to 5 min, the rate of ascorbate oxidation was

documented. The enzyme specific activity of ascorbate peroxidase was estimated as mmol ascorbate oxidized mg^{-1} (protein) min^{-1}.

Hydrogen peroxidase (H_2O_2): Measurement of H_2O_2 content in leaf sample was done as per the method described by Jana and Choudhuri (1981). 200 mg of fresh leaf sample was homogenized in 50 mM phosphate buffer (pH 6.5) to extract H_2O_2 and centrifugation of the homogenized sample at 8000 × g for 20 min was followed. To 3 ml of the supernatant, 1 ml of 0.1% titanium sulphate was added followed by centrifugation at 8000 × g for 15 min. The measurement of intensity of the yellow color mixture was done at 410 nm. Using the extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}, the H_2O_2 concentration was measured and expressed as µmol g^{-1} FW.

Electrolytic leakage: EL was estimated with the help of a portable conductivity meter (CM-180, Elico, India). The EL was calculated by the equation:

EL (%) = x/y × (100).

Results and Discussion

Analysis of variance showed significant differences among the genotypes for the fifteen characters studied. Analysis of variance showed significant difference among the genotypes for the different characters at 0.1% and 5% significance. The mean sum of squares due to genotype for different characters are presented in Table 1.

The variance measures the variation within a particular trait. But it does not provide any real measure for comparison of variance between different traits. The term "Coefficient of Variation (CV)" truly provides a relative measure of variance among different traits. In general, estimates of phenotypic coefficient of variation (PCV) were found to be higher than their corresponding genotypic coefficient of variation (GCV), this was due to environmental component, which was being added to GCV. The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for all the fifteen characters were presented in Table 2 Similar observations in tomato were also reported Singh *et al.* (2006) and Hayadar *et al.* (2007). According to Sivasubramanian and Madhavamenon (1973), genotypic coefficient of variation (GCV) and phenotypic coefficient

Table 1 : Analysis of variance for fifteen physiological, nutritional and biochemical morphological characters in tomato.

S. no.	Source of variation/ characters	Mean sum of squares		
		Replication D.f=2	Treatments D.f=29	Error D.f=58
1.	Chlorophyll A	0.17	1.47**	0.08
2.	Chlorophyll B	0.04	0.09**	0.02
3.	Leaf Area Index	8.74	7.69**	4.22
4.	TSS (°Brix)	3.01	4.74**	1.46
5.	pH	0.01	0.06**	0.01
6.	Beta carotene	0.01	0.05**	0.01
7.	Lycopene	0.36	4.74**	0.17
8.	Ascorbic acid	0.456	15.36**	0.22
9.	Acidity	0.02	0.04**	0.01
10	Proline	0.37	2.21**	0.21
11	SOD	0.161	396.91**	0.08
12	Peroxide (POX)	0.26	8332.70**	0.13
13	Ascorbate peroxidase (APX) (µmol)	4.962	271.88**	2.40
14	Hydrogen Peroxide	7.5	2277.57**	3.63
15	Electrolytic Leakage	22.54	1707.32**	10.88

* Significant at 1%, ** Significant at 5%.

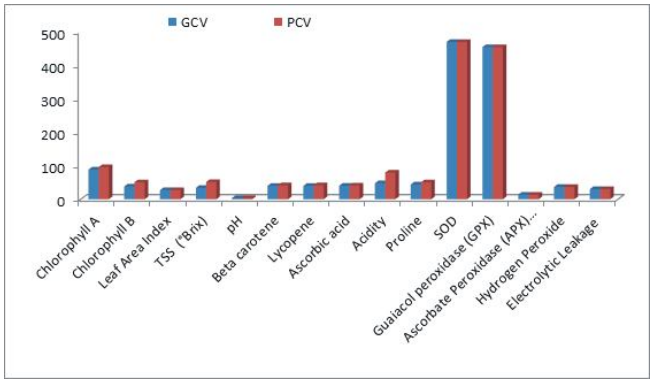

Fig. 1 : Histogram depicting estimates of GCV and PCV for morphological traits of tomato.

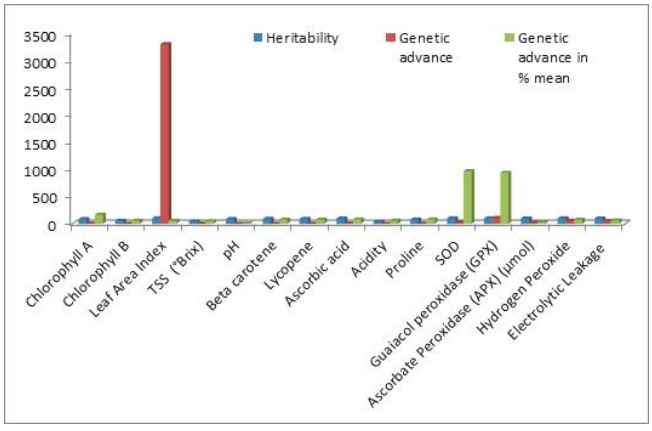

Fig. 2 : Histogram depicting estimates of Heritability, G. A. and G. A. as mean (%) for morphological traits traits of tomato.

Table 2 : Estimates of Genetic variability of tomato genotypes for fifteen physiological, nutritional and biochemical parameters.

	Characters	Grand mean ±SE	Range	Coefficient of Variation		Coefficient of variability		Heritability advance	Genetic	Genetic advance in % mean
				Genotypic	Phenotypic	Genotypic	Phenotypic			
Physiological and nutritional traits	Chlorophyll A	0.78± 0.24	4.11- 0.00	0.47	0.55	88.34	95.97	84.74	1.29	167.53
	Chlorophyll B	0.42± 0.11	0.81-0.0	0.03	0.04	37.81	50.40	56.27	0.24	58.43
	Leaf Area Index	5889.14± 1.68	8527.0-2326.0	2565486.75	2565490.98	27.18	27.18	100.00	3299.53	55.99
	TSS (°Brix)	3.12± 0.98	4.44-0.00	1.10	2.55	33.64	51.28	43.03	1.42	45.45
	pH	4.60± 0.05	4.87-4.33	0.018	0.021	2.88	3.13	84.94	0.25	5.47
	Beta carotene	0.35 ±0.04	0.49-0.00	0.019	0.021	39.73	41.64	91.04	0.27	78.10
	Lycopene	3.12± 0.34	4.44-0.00	1.52	1.70	39.63	41.85	89.68	2.41	77.31
	Ascorbic acid	5.61± 0.38	7.99-0.00	5.05	5.27	40.09	40.96	95.82	4.53	80.85
	Acidity	0.19± 0.10	0.46-0.00	0.008	0.022	47.81	79.57	36.10	0.11	59.18
Biochemical and additional traits	Proline	1.87 0.37	3.84-0.00	0.67	0.87	43.98	50.23	76.69	1.48	79.34
	SOD	2.53± 0.23	0.82-0.00	132.28	132.36	469.00	469.14	99.94	23.69	965.86
	Guaiacol peroxidase (GPX)	11.88± 0.29	3.80-0.00	277.75	277.7	453.77	453.78	100.00	108.56	934.75
	Ascorbate Peroxidase (APX) (µmol)	71.99± 1.26	84.56-45.7	89.83	92.22	13.22	13.40	97.40	19.27	26.89
	Hydrogen Peroxide	75.68± 1.55	96.23-0.00	757.98	761.60	36.39	36.47	99.52	56.58	74.78
	Electrolytic Leakage	78.80± 2.69	97.90-0.00	565.48	576.36	29.99	30.28	98.11	48.52	61.19

of variation (PCV) have been classified into low when less than 10%, moderate when 10-20% and high when greater than 20%. Wide range of genotypic and phenotypic coefficient of variation (GCV & PCV) was observed for the characters ranging from SOD (469.00 and 469.14) to pH (2.88 and 3.13). High magnitude of GCV and PCV were recorded for chlorophyll a (88.34 & 95.97), chlorophyll b (37.81 & 50.40), leaf area index (27.18 & 27.18), TSS (°Brix) (33.64 & 51.28), beta carotene (39.73 & 41.64), lycopene (39.63 & 41.85), ascorbic acid (40.09 & 40.96), acidity (47.81 & 79.57), proline (43.98 & 50.23), SOD (469.00 & 469.14), Guaiacol peroxidase (GPX) (453.77 & 453.78), hydrogen peroxide (36.39 & 36.47) and electrolytic leakage (29.99 & 30.28). The results are in line with the results reported earlier by Meena *et al.* (2015), Singh *et al.* (2015), Sunil kumar *et al.* (2016), Panchbhaiya *et al.* (2018), Anuradha *et al.* (2020), Sathiyavarsha *et al.* (2023) and Sairam *et al.* (2024). The results were indicated the similar trend to the results of Golani *et al.* (2007); Javed *et al.* (2022) and Rasheed *et al.* (2023). Whereas moderate estimates were observed for Ascorbate Peroxidase (APX) (µmol) (13.22 & 13.40). Whereas low estimates were observed for pH (2.88 & 3.13). The reliability of genotype identification by phenotype is improved by heritability and genetic advancement. The broad sense of heritability was found high for all the six characters under study. According to Johnson *et al.* (1955), heritability estimates were classified into low, when less than 30%, moderate when 30-60% and high when greater than 60%. In the present investigation, the heritability estimates were found to be high (more than 60%) for chlorophyll a (84.74), leaf area index (100.00), pH (84.94), beta carotene (91.04), lycopene (89.68), ascorbic acid (95.82),

proline (76.69), SOD (99.94), guaiacol peroxidase (GPX) (100.00), ascorbate peroxidase (APX) (µmol) (97.40), hydrogen peroxide (99.52) and electrolytic leakage (98.11). Whereas moderate estimates were observed for chlorophyll B (56.27), TSS (°Brix) (43.03) and acidity (36.10). The results of Golani *et al.* (2007), Sunilkumar *et al.* (2016), Anuradha *et al.* (2020) and Rasheed *et al.* (2023) mimic the present findings. However, when the estimate of expected genetic advance accompanies heritability, then the prediction of genetic gain under selection is more accurate (Johnson *et al.*, 1955). The classification of genetic advance as per cent of mean has been given by Johnson *et al.* (1955) as low, when less than 10%, moderate when 10-20% and high when greater than 20%. In the present investigation, the genetic advance in % mean estimates were found to be high for chlorophyll a (167.53), chlorophyll b (58.43), leaf area index (55.99), TSS (°brix) (45.45), beta carotene (78.10), lycopene (77.31), ascorbic acid (80.85), acidity (59.18), proline (79.34), sod (965.86), guaiacol peroxidase (GPX) (934.75), ascorbate peroxidase (APX) (µmol) (26.89), hydrogen peroxide (74.78) and electrolytic leakage (61.19). Whereas pH (5.47) showed low genetic advance in % mean. Genetic advance for various characters are presented in Table 2 and noticed that high genetic advance was recorded for leaf area index (3299.53), SOD (23.69), guaiacol peroxidase (GPX) (108.56), hydrogen peroxide (56.58) and electrolytic leakage (48.52). Whereas Ascorbate Peroxidase (APX) (µmol) (19.27) showed moderate genetic advance. The genetic advance estimates were found to be lowest for Chlorophyll A (1.29), Chlorophyll B (0.24), TSS (°Brix) (1.42), pH (0.25), Beta carotene (0.27), Lycopene (2.41), Ascorbic acid (4.53), Acidity (0.11) and Proline (1.48). This indicates that additional genetic influences predominate in the expression of these characters. The values arrived in this experiment are matching with the records of Golani *et al.* (2007), Sunilkumar *et al.* (2016), Anuradha *et al.* (2020), Rahimi *et al.* (2022) and Srinivasulu *et al.* (2024).

Conclusion

The study revealed significant variability and elevated heritability for all quality traits examined, highlighting their potential for genetic improvement. High (>20) magnitude of GCV and PCV were recorded for SOD (469.00 and 469.14). The heritability estimates were found to be high (more than 60%) for leaf area index and guaiacol peroxidase (GPX) (100.00). Whereas the genetic advance estimates were found to be high for leaf area index (3299.53). Whereas the genetic advance in % mean estimates were found to be high for SOD (965.86).

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