



Evaluation of Soybean (*Glycine max* L.) Genotypes on the Basis of Biochemical Contents and Anti-oxidant Enzyme Activities

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ABSTRACT

Background: Soybean is an important leguminous crop. Abnormal weather has played an enormous role in the strident decline in crop yields. Drought is considered as a significant abiotic factor responsible for yield reduction in soybean.

Methods: The present work was carried out in order to screen soybean genotypes for their drought tolerance ability by means of different biochemical and antioxidant enzymatic activities responses.

Conclusion: On the basis of biochemical parameters and anti-oxidant enzymatic activities, soybean genotype viz., RVS-211-77, RVS-211-75, NRC-7, SL-96, NRC-136, AMS100-39, SL-96, RVS-2012-01, RVS-211-73 and JS97-52 have been identified with better performance and can be used as parents for further crop improvement programme to breed drought tolerant variety.

Key words: Anti-oxidant, Breeding, Crop improvement, Drought, Soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a vital and inexpensive protein and oil seed crop. Therefore, in numerous emerging nations, it is used as an imperative constituent of human foods and animal feed stuff. Its seed comprehend near 20-22% edible oil, 32-35% protein and 35-38% carbohydrates (17% of that is alimetal fiber) and around 5% ash in addition to minerals and vitamins (Tripathi and Tiwari, 2004; Tiwari and Tripathi, 2005; Mishra *et al.*, 2020; Upadhyay *et al.*, 2020a; Mishra *et al.* 2021a). Soybean oil plays a vital part in nourishment of human as it has better extent of indispensable unsaturated fatty acids like omega-3, omega-6 and omega-9.

Plant growth and yield of soybean are decidedly diminished by numerous biotic and abiotic factors. Drought is prime environmental stress conditions that diminutions crop production and excellence, therefore posturing a thoughtful hazard to crop production (Mishra *et al.*, 2021b; Upadhyay *et al.*, 2020b). Stress constrains the synthesis of photosynthetic harvests due to reductions in leaf photosynthetic dimensions (Anjum *et al.*, 2011) and the acceleration of leaf wilting and ageing (Pinheiro *et al.*, 2005).

Drought influences plant growth through oxidative stress tracked by illnesses in diverse biological progressions. Responsive oxygen species (ROS) distresses antioxidant enzyme bustle and waning photosynthetic action and stomatal opening. Screening of drought tolerant genotypes on the basis of different biochemical parameters and anti-enzymatic activities may deliver a sordid for the development of new plant varieties applying conventional along with molecular breeding tactics to fight drought. The current investigation was executed to monitor drought

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tolerant soybean genotype (s) based on manifestation of different biochemical parameters and antioxidant enzymes activities.

MATERIALS AND METHODS

The present study was consisted of 60 soybean genotypes (Table 1) with conflicting reactions to drought viz., susceptibility and tolerance. The field trial was department at the investigational field, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, India during July 2019 to May 2020 in randomized block design in three rows with two replications and row to row distance was kept 30 cm. Data were recorded after 70 days of sowing from five random selected plants of each line and replication for diverse biochemical parameters. The data were investigated according to method proposed by Snedecor and Cochran (1967).

Biochemical parameters

Photosynthetic pigments

Photosynthetic pigments were estimated by adopting method of Arnon (1949). Measurement of photosynthetic pigment was accomplished employing UV-VIS spectrophotometer for documenting absorbance at 470, 645 and 663 nm. The magnitude of chlorophyll a, chlorophyll b and total chlorophyll was computed by following formulae:

$$\text{Chl}_a = (12.7 \times \text{Abs}_{663}) - (2.6 \times \text{Abs}_{645}) \times 10 \text{ ml of acetone/} \\ 100 \text{ mg leaf tissue.}$$

$$\text{Chl}_b = (22.9 \text{ Abs}_{645}) - (4.68 \text{ Abs}_{663}) \times 10 \text{ ml of acetone/} \\ 100 \text{ mg leaf tissue.}$$

$$\text{Chl}_{a+b} = (22.9 \text{ Abs}_{645}) - (4.68 \text{ Abs}_{663}) \times 10 \text{ ml of acetone/} \\ 100 \text{ mg leaf tissue.}$$

Estimation of sugar (mg g⁻¹ fresh weight) and proline (unit) contents

Sugar quantity was computed by mentioning glucose standard curve as adopted by Kachare (2017) in soybean. Free proline content in leaves was determined according to the method recommended by Bates *et al.* (1973).

Membrane stability index

Membrane stability index (MSI) was computed by using following formula.

$$\text{Membrane Stability Index} = [1 - \{C_1/C_2\}] \times 100$$

C_1 = Electrical conductivity of water containing the leaf sample in set one.

C_2 = Electrical conductivity of water containing the leaf sample in set two.

Antioxidant enzyme activity (catalase, glutathione reductase and peroxidase)

The actions of diverse antioxidant enzymes have been determined employing spectrophotometer. Estimation of Ascorbate peroxidase (APX) activity was done following the method of Nakano and Asada (1981). In the first step diluted enzyme extract (20 μ l) was added in 50 mM potassium phosphate buffer (880 μ l) containing 0.5 mM ascorbate. In the second step 1 mM H₂O₂ (100 μ l) was added to start the reaction. Further the absorbance was recorded at 15 s interval for 2 min at 290 nm. Catalase activity was estimated by the UV method of Aebi (1983) with some modifications. 100 μ l of diluted enzyme isolated from leaves was mixed with 800 μ l of 50 mM potassium phosphate buffer for the experiment (pH 7.0). 100 μ l of 100 mM hydrogen peroxide was used to start the reaction. The change in absorbance was measured at 240 nm at 15 second intervals. GR activity was measured according to the method of Smith *et al.* (1988) with needed modifications. Diluted enzyme extract (25 μ l), 5, 5'-dithio-bis (2-nitrobenzoic acid) (250 μ l), 1mM EDTA (20 μ l) and 0.2 mM oxidized glutathione (100 μ l) were added in 175 μ l

of 50 mM potassium phosphate buffer (pH 7.6). In the next step 50 μ l of 5 mM NADPH added to the reaction mixture to initiate GR activity. Changes in absorbance at 412 nm were recorded at 15 s interval. Guaiacol peroxidase (POX) activities were analyzed the method adopted by Kachare (2017).

RESULTS AND DISCUSSION

Biochemical analysis

The analysis of variance presented in Table 2 clearly indicated existence of substantial amount of variations among 60 soybean genotypes included in the present study for all biochemical parameters investigated. Chlorophyll is the core photosynthetic pigments that decide biomass of plants. Generally, the magnitude of chlorophyll content in leaves regulates the rate of photosynthesis. Drought stress constrains the photosynthesis by means of instigating alterations in chlorophyll content. Total Chlorophyll content in mg/ml varied significantly in range of 36.58-58.33 mg/ml in present investigation, maximum with genotype RVS 2011-75 while the minimum was in JS20-53*JS20-34. Anjum *et al.* (2011) displayed a decline in the quantity of chlorophyll because of forfeiture of chloroplast membranes in drought susceptible genotypes. Analogous reduction in chlorophyll levels in many other plant species as well as soybean (Zhang *et al.*, 2007; Mishra *et al.*, 2021c), chickpea (Sahu *et al.*, 2020) and pearl millet (Choudhary *et al.*, 2021).

Soluble sugars are the key osmotic modification constituents and therefore are significant pointers of tolerance/resistance in genotypes. Total sugar in mg/g varied significantly in range of 2.4-5.9 mg/g with maximum in genotype RVS2011-77 trailed by genotypes RVS2011-73 and RVS 2011-10, while the minimum was perceived in genotype CAT87 and JS335 tracked by genotype PK885. Genotypes pursuing higher sugar content might be drought tolerant as reported earlier by Kachare *et al.* (2019) and Mishra *et al.* (2021c). The augmentations in MSI designate the lessening of lipid peroxidation with oxidative bursts under water stress conditions. Genotype PS1611 exhibited maximum membrane stability (MI) tracked by genotypes viz., JS20-90 and JSM240*SL517 whereas, it was displayed by genotype AMS-MB 5-18 intimately pursued by genotypes Hardee and Gaurav.

Proline is trusted as an authoritative drought tolerance pointer. Proline content in μ g/g varied meaningfully between 65.31-107.27 μ g/g with utmost in genotype EC 602288 (107.27) followed by genotypes JS97-52 (105.45), EC538828 (101.88) and NRC7 (100.25), more than 100 μ g/g. However, the minimum was observed in genotype JS2009*PS1475 (65.31) trailed by genotypes JS2063*JS95-60 (67.63) and SL 525 (67.9). Genotypes exhibiting higher proline content may have drought tolerance as suggested by Kachare *et al.* (2019) and Mishra *et al.* (2021c) as they

Table 1: List of soybean genotypes with their source.

Genotypes	Source/Origin	Parentage
RVS2011-76	RVSKVV, Gwalior	JS20-29 × JSM275
RVS2011-35	RVSKVV, Gwalior	JS 335 × PK 1042
RVS 28	RVSKVV, Gwalior	JS20-29 × JS20-22
RVS2011-10	RVSKVV, Gwalior	JS -335 × PK 1042
RVS2012-15	RVSKVV, Gwalior	JSM240 × SL517
RVS2011-77	RVSKVV, Gwalior	JS20-30 × JS93-05
RVS2011-73	RVSKVV, Gwalior	JS95-60 × JSM110
RVS2011-74	RVSKVV, Gwalior	JS20-31 × JS335
RVS2011-21	RVSKVV, Gwalior	JS97-52 × JS20-09
RVS2012-01	RVSKVV, Gwalior	JS97-52 × JSM238
RVS2011-32	RVSKVV, Gwalior	JS335 × PK-1042
RVS2011-04	RVSKVV, Gwalior	JSM226 × JS20-09
RVS2011-75	RVSKVV, Gwalior	JS20-34 × JS20-22
NRC136	NRCS, Indore	JS 97-52 × NRC 37
NRC37	NRCS, Indore	Gaurav × Punjab1
NRC7	NRCS, Indore	Selection from S-69-96
SL688	PAU, Ludhiana	PK416 × SL317
SL958	PAU, Ludhiana	SL 525 × SL 706
SL96	PAU, Ludhiana	Botato × JS 3
SL953	PAU, Ludhiana	-
SL983	PAU, Ludhiana	SL-525 × PK-1368
SL995	PAU, Ludhiana	
SL525	PAU, Ludhiana	PK 416 × PK 1023
SL1074	PAU, Ludhiana	PK-1223 × SL(E) 14
EC602288	Exotic collection	Exotic collection
EC538828	Exotic collection	Exotic collection
EC46728	Exotic collection	Exotic collection
Young	China	Exotic collection
C-2797		-
PK472	GBPUAT, Pantnagar	Hardee × Punjab-1
Bragg	USA	Jackson × D 49-2491
Hardee	USA	D 49-772 × Improved Pelican
Gaurav	JNKVV, Jabalpur	D 60-9647 × EC 7034
DS3106	New Delhi	-
DS3105	New Delhi	-
JSM240*SL517	JNKVV, Jabalpur	JSM240*SL517
HIMSO-1685	JNKVV, Jabalpur	
PS1225	GBPUAT, Pantnagar	PK 515 × PK 327
CAT87		Not known selection
RSC1052	Raipur	-
BAUS102	Ranchi	-
PK885	GBPUAT, Pantnagar	
Shivalika	JNKVV, Jabalpur	Selection from PK 7355
AUKS-174		
SP-37	Not known selection	Not known selection
JS335	JNKVV, Jabalpur	JS 78-77 × JS 71-5
AMS475	PDKV, Akola	Mutant of JS93-05
AMS243	PDKV, Akola	Mutant of Bragg
AMS-MB5-18	PDKV, Akola	Mutant of Bragg
JS20-09*PS1475	JNKVV, Jabalpur	JS20-09*PS1475
JS20-63*JS95-60	JNKVV, Jabalpur	JS20-63*JS95-60
JS20-53*JS20-34	JNKVV, Jabalpur	JS20-53*JS20-34
JS20-90	JNKVV, Jabalpur	JS 97-52 × JS 95-56
JS97-52	JNKVV, Jabalpur	PK 327 × L129
JS21-17	JNKVV, Jabalpur	JS 20-63 × JS 95-60
JS20-78	JNKVV, Jabalpur	JS 98-61 × EC-333922
JS93-05	JNKVV, Jabalpur	Secondary selection from PS 73-22
AMS100-39	PDKV, Akola	Mutant of JS93-05
RVS2001-04	RVSKVV, Gwalior	JS93-01 × EC390981
PS1611	PAU, Ludhiana	-

Table 2: Mean performance of different biochemical parameters and antioxidant enzymes activities of soybean genotypes.

Genotypes	Total chlorophyll content (mg/ml)	Total sugar (mg/g)	MSI (%)	Proline content	Ascorbate peroxidase (unit/mg protein)	Catalase (unit/mg protein)	Glutathione reductase (unit/mg protein)	Guaiacol peroxidase (unit/mg protein)
RVS 2011-76	51.84	4.8	45	94.06	0.78	0.81	0.30	0.59
RVS 2011-35	43.06	4.6	44	73.32	0.42	0.32	0.21	0.77
RVS 28	45.93	5.6	43	93.93	0.59	0.58	0.30	0.82
RVS 2011-10	48.03	5.7	47	70.88	0.77	0.43	0.32	0.39
RVS 2012-15	44.62	5.3	51	76.33	1.54	0.49	0.36	0.72
RVS 2011-77	49.14	5.9	52	79.83	0.59	0.45	0.41	0.86
RVS2011-73	47.68	5.8	54	83.33	0.43	1.21	0.34	0.49
RVS 2011-74	40.97	5.1	55	70.83	0.31	0.64	0.48	0.51
RVS 2011-21	42.53	5.3	50	81.43	0.32	0.65	0.61	0.71
RVS 2012-01	48.45	3.2	49	77.23	1.77	0.54	0.30	1.9
RVS 2011-32	41.7	3.1	41	86.38	0.31	0.53	0.38	0.9
RVS 2011-04	43.73	2.6	39	81.28	0.34	0.74	0.50	0.45
RVS 2011-75	58.33	2.9	53	84.38	0.42	0.77	0.31	0.73
NRC136	38.7	2.9	44	81.00	0.51	0.88	0.34	0.48
NRC37	43.37	2.6	41	86.88	0.58	0.91	0.45	0.71
NRC7	45.67	4.9	38	100.25	0.39	0.48	0.91	1.41
SL 688	49.11	3.1	38	72.44	0.3	0.67	0.27	0.46
SL 958	45.55	3.4	46	79.20	0.48	0.71	0.21	0.51
SL 96	51.15	5.1	50	87.56	2.08	0.98	0.76	2.35
SL 953	45.75	2.9	44	73.38	0.46	0.46	0.6	0.62
SL 983	39.86	2.7	39	68.72	0.47	0.51	0.61	0.61
SL 995	50.17	3.8	50	74.63	0.38	0.7	0.50	0.42
SL 525	46.1	3.5	37	67.90	0.29	0.44	0.45	0.79
SL 1074	46.3	3.7	50	85.57	0.24	0.31	0.34	0.3
EC 602288	46.84	3.9	44	107.27	0.22	0.41	0.61	0.53
EC 538828	42.19	3.2	54	101.88	0.53	0.6	0.56	0.78
EC 46728	46.29	3.3	47	77.83	0.41	0.54	0.48	0.55
Young	44.54	2.9	44	90.92	0.39	0.36	0.39	0.41
C-2797	42.48	2.4	33	78.83	0.33	0.67	0.37	0.62
PK 472	41.84	4.3	54	91.77	0.27	0.38	0.30	0.56
Bragg	45.72	4.7	50	99.81	0.4	0.49	0.51	0.87
Hardee	48.14	4.1	30	95.37	0.37	0.45	0.54	0.72
Gaurav	51.51	4	30	81.83	0.3	0.53	0.43	0.91
DS 3106	47.52	3.7	42	72.18	0.41	0.52	0.42	0.53
DS 3105	49.3	3.9	51	68.50	0.85	0.59	0.49	0.61
JSM240*SL517	44.41	3.4	58	80.64	0.76	0.48	0.60	0.59
HIMSO-1685	48.11	3.5	33	78.98	0.8	0.67	0.58	0.63
PS 1225	51.3	3.1	41	84.65	0.78	0.61	0.59	0.66
CAT 87	52.3	2.4	53	90.45	0.81	0.38	0.23	0.82
RSC 1052	49.67	2.8	50	75.15	0.69	0.47	0.48	0.88
BAUS 102	52.51	2.6	52	80.84	0.59	0.46	0.49	0.91
PK 885	55.32	2.5	35	69.12	0.67	0.61	0.80	0.83
Shivalika	50.56	3.2	36	80.63	0.65	0.4	0.50	0.81
AUKS-174	49.21	3.6	50	90.25	0.89	0.62	0.61	0.7
SP-37	51.62	3.1	52	85.91	0.71	0.41	0.65	0.58
JS 335	53.76	2.4	50	88.40	0.74	0.48	0.75	0.45
AMS 475	51.8	2.8	41	89.33	0.43	0.35	0.77	0.71
AMS 243	54.6	4.1	47	82.23	0.61	0.72	0.83	0.52

Table 2: Continue...

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AMS-MB 5-18	46.05	4.7	28	70.39	0.5	0.74	0.79	0.59
JS2009*PS1475	46.34	5.2	48	65.31	0.67	0.41	0.81	0.72
JS2063*JS95-60	49.25	4.2	35	67.63	0.58	0.55	0.76	0.59
JS20-53*JS20-34	36.58	3.6	50	68.13	0.77	0.88	0.67	0.46
JS 20-90	48.59	2.8	64	69.74	0.42	0.52	0.51	0.31
JS 97-52	55.07	3.2	50	105.12	0.46	0.66	0.53	0.31
JS 21-17	48.19	2.8	44	90.52	0.38	0.41	0.42	0.53
JS 20-78	58.28	2.9	78.53	78.53	0.78	0.4	0.53	0.64
JS 93-05	53.94	3.1	87.98	87.98	0.51	0.67	0.66	0.22
AMS100-39	54.53	5.5	82.92	82.92	0.66	0.56	0.73	1.92
RVS 2001-04	48.89	3.1	90.25	90.25	0.62	0.71	0.66	0.64
PS1611	50.39	3.9	75.43	75.43	0.68	0.81	0.26	0.74
Minimum	36.58	2.4	28	65.31	0.22	0.31	0.21	0.22
Maximum	58.33	5.9	65	107.27	2.08	1.21	0.91	2.35
Mean	47.92	3.72	45.52	82.09	0.59	0.58	0.51	0.71
SE	2.21	0.35	2.65	2.44	0.05	0.06	0.04	0.08
CD _{0.05}	6.24	0.99	7.49	6.89	0.15	0.13	0.11	0.22

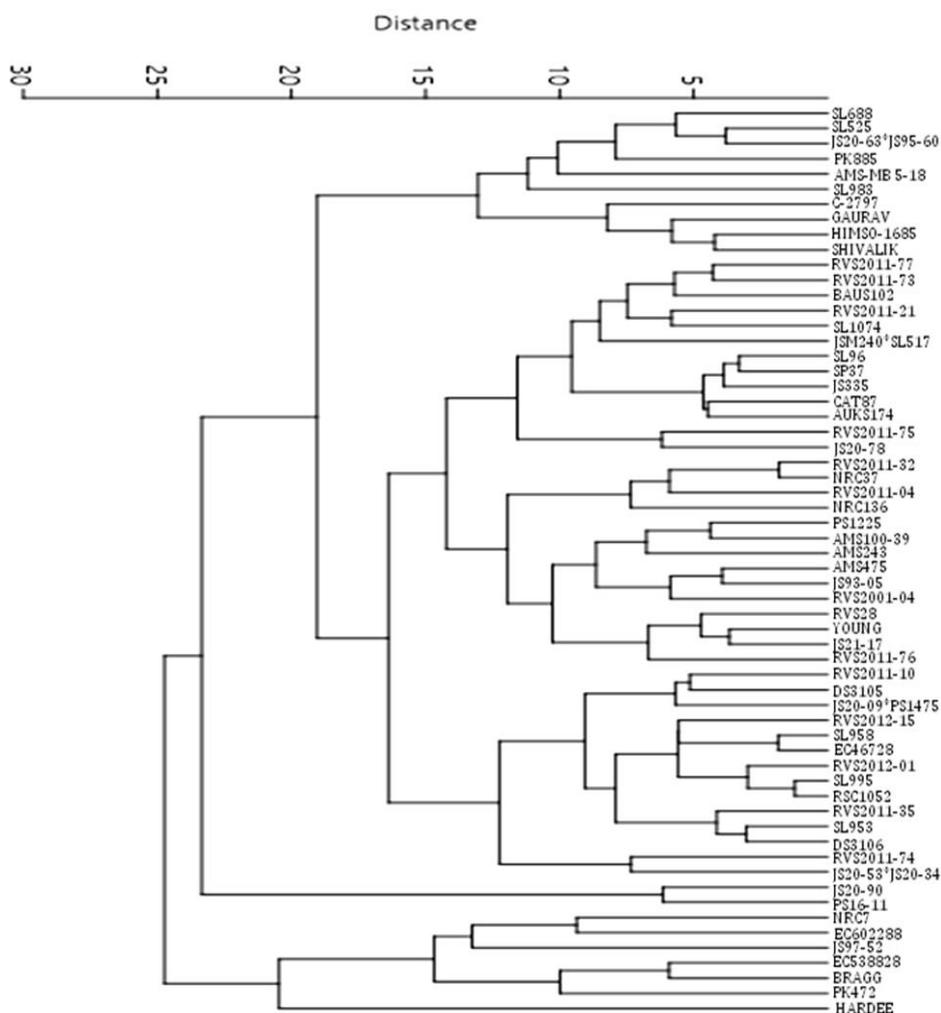


Fig 1: Dendrogram showing relationship among genotypes based on different biochemical contents (Total chlorophyll, proline, total sugar and MSI).

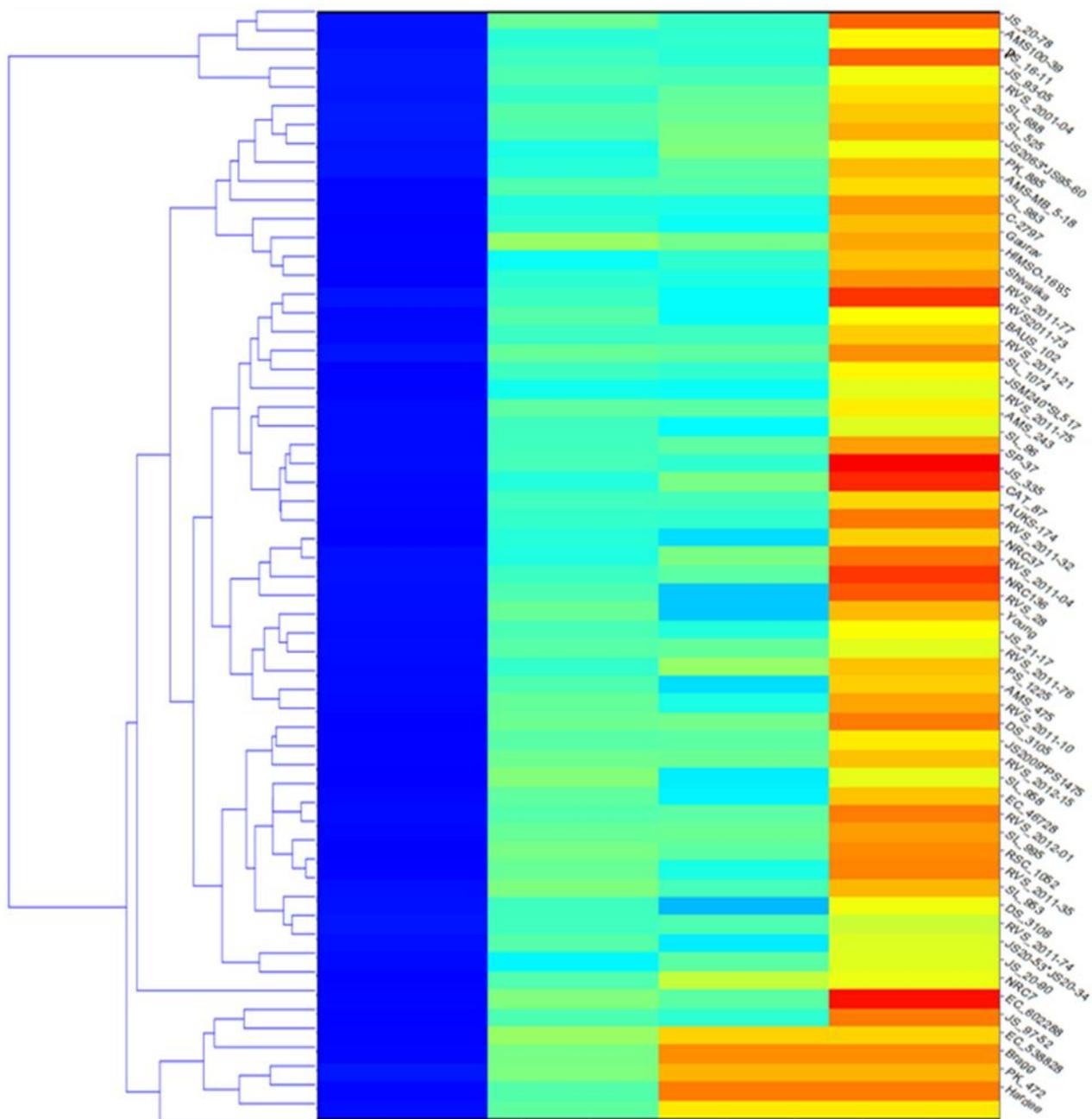


Fig 2: Clustering pattern of different genotypes for different biochemical contents.

also concluded during their study on screening of soybean genotypes. It may be perhaps due to increased proline content maintains cell water level under drought (Choudhary *et al.*, 2021).

Cluster analysis of different biochemical parameters (Total chlorophyll content, proline content, total sugar and MSI)

On the basis of dendrogram (Fig 1), genotypes formed two clusters. Major cluster consisted 53 genotypes while minor cluster had only 7 genotypes including NRC7, EC602288,

JS97-52, EC538828, Bragg, PK472 and Hardee. Major group further divided into two groups. Major sub group consisted 51 genotypes, however minor sub group had two genotypes, *namely* JS 20-90 and PS 1611. Major sub group further divided into two groups. Major group consisted 41 genotypes, however minor group had 10 genotypes including SL688, SL525, JS2063*JS95-60, PK885, AMS-MB 5-18, SL983, C-2797, Gaurav, HIMSO-1685 and Shivalika. Major group consisted 41 genotypes *viz.*, RVS2011-77, RVS2011-73, BAUS102, RVS2011-21,

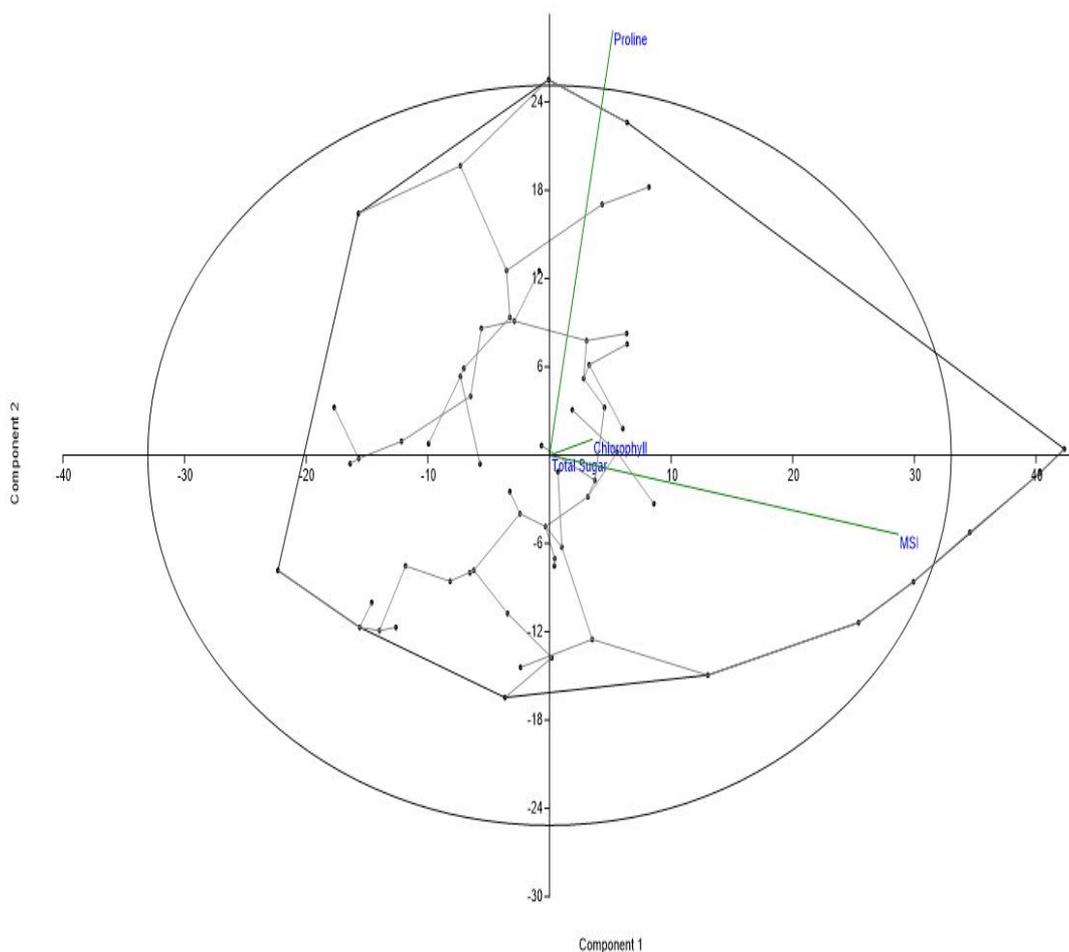


Fig 3: Principal component analysis of biochemical contents.

SL1074, JSM240*SL517, SL96, SP-37, JS335, CAT87, AUKS-174, RVS2011-75, JS20-78, RVS2011-32, NEC 37, RVS2011-04, NRC136, PS1225, AMS100-39, AMS243, AMS475, JS93-05, RVS2001-04, RVS28, Young, JS21-17, RVS2011-76, RVS2011-10, DS3105, JS2009* PS1475, RVS2012-15, SL958, EC46728, RVS 2012-01, SL 995, RSC 1052, RVS 2011-35, SL 953, DS 3106, RVS 2011-74 and JS20-53*JS 20-34.

Biochemical analysis based hierarchical cluster analysis

Bestowing to the hierarchical cluster investigation and the gratified values (Table 2), the active countenance outline was governed and is displayed in Fig 1. Multivariate examination owing to diversity was executed through the UPGMA. The average worth of biochemical parameters the dendrogram was discriminated into two major clusters *i.e.*, I and II. Cluster I and II divided into subclusters. These clusters further subdivided into minor clusters. Genotypes JS-20-78, AMS-100-99, PK-472 and Hardee fallen in a separate group.

Biochemical parameters based principal component analysis (PCA)

Principal component analysis was conducted by taking biochemical variables concurrently. The PCA correlation illustrated that which variety possessed higher and lower content occupying unique position towards the graph (Fig 2). In these four variables, total sugar content had highest variability (58.8%).

Antioxidant enzymes activities

Antioxidative enzymes perform a key part in defending plants from the damaging consequences of drought stress. Plants change enzymatic devices encompassed in the enzymatic rummaging of Reactive Oxygen Species (ROS) for instance APX, CAT, GR and PDX. Lessons on soybean disclosed that stress tolerant plants are usually bestowed with competent antioxidant protection arrangement (Vasconcelos *et al.*, 2009). Earlier it was also noticed that CAT and GR had a higher ROS hunting action than APX, so, it could be assumed that CAT and GR are the most significant ROS foraging enzymes in soybean owing to their involvement in

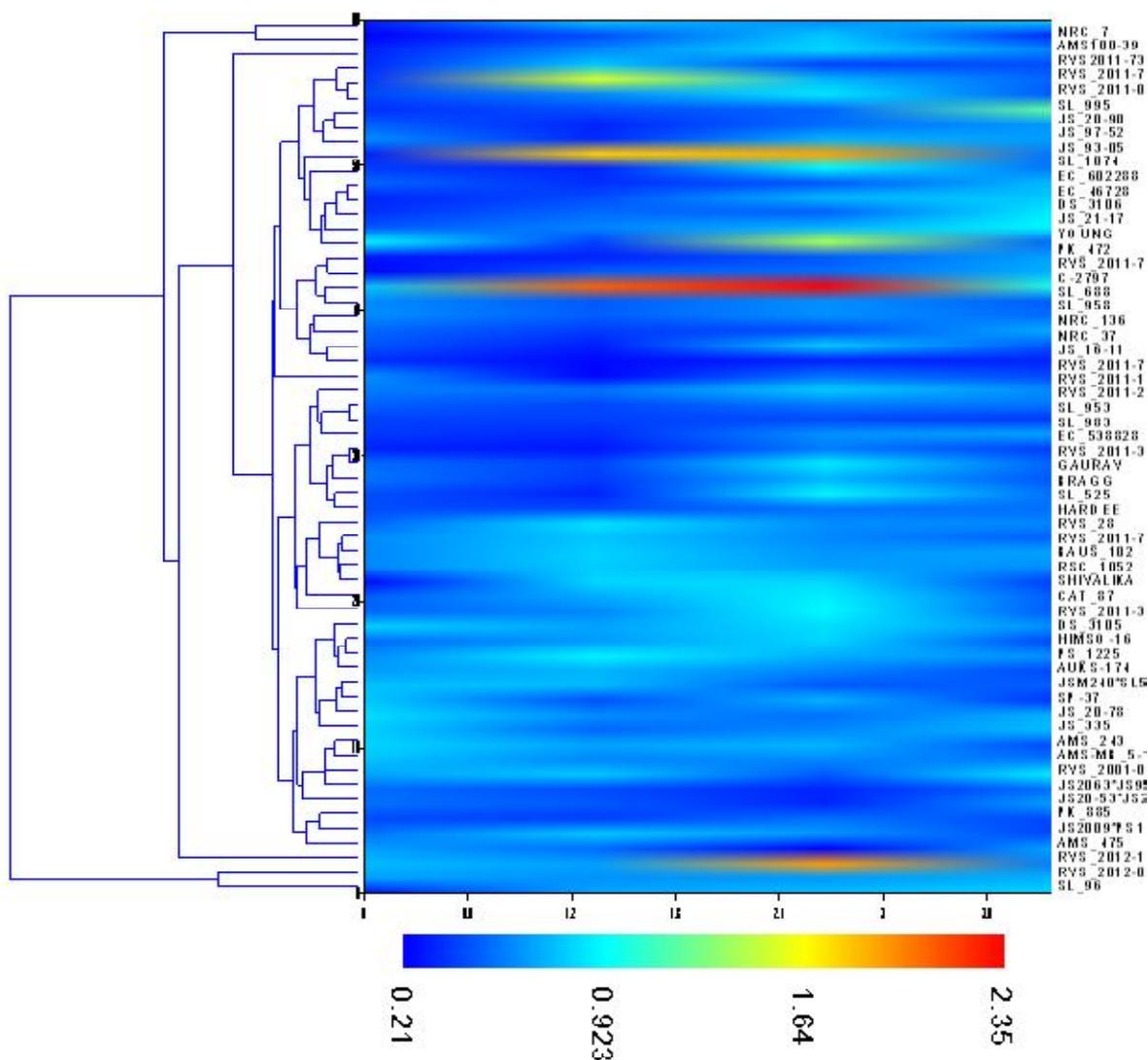


Fig 5: Clustering pattern of soybean genotypes for antioxidant enzymatic activities.

Cluster analysis of pooled antioxidative enzymes data

In antioxidant enzymes data based dendrogram the genotypes were divided into two clusters *i.e.*, major cluster and minor cluster. The major cluster contained 58 genotypes while the minor cluster had only 2 genotypes *viz.*, RVS 2012-01 and SL 96. Major group further subdivided into two groups major sub group 1 and minor sub group 2. Major sub group 1 consisted 51 genotypes, however minor sub group had only 2 genotypes, namely NRC-7 and AMS 100-39. Minor sub group further subdivided into two parts. RVS-2011-73 genotypes came in outgroup of the cluster.

Antioxidant activities estimate based hierarchical cluster analysis

Dynamic expression profile was constructed on the basis of hierarchical cluster analysis and the content values presented in Fig 3. Multivariate analysis owing to diversity

was executed using the UPGMA. The mean value of antioxidant enzymes activities of different genotypes depicting in each cluster was presented in the generated dendrogram and distinguished into two major clusters (I and II). Cluster I and II divided into subclusters. These clusters further subdivided into minor clusters (Fig 4 and Fig 5). Cluster I consisted NRC-136 and AMS100-39 genotypes as an outer group and cluster II also consisted two genotypes *viz.*, RVS 2012-01 and SL-96 as an outgroup.

Antioxidant activities based principal component analysis (PCA)

Principal component analysis (PCA) was drawn by considering biochemical variables instantaneously. The decoration of variations exemplified by the PCA designated by correlation coefficients explained for pair-wise connotation of the traits. The PCA correlation illustrated that genotype

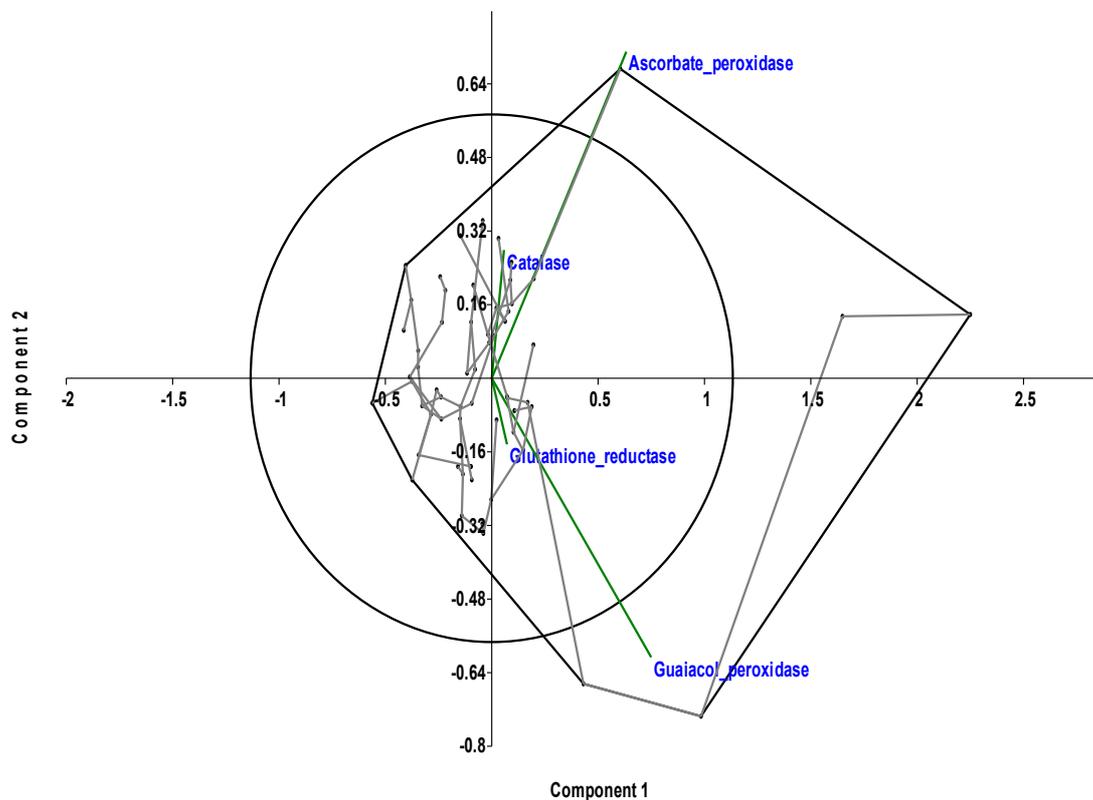


Fig 6: Principal Component analysis of antioxidant enzymatic activities of genotypes.

possessed higher and lower antioxidative content occupying unique position towards the graph (Fig 6). In these four variables, Glutathione reductase content had highest variability (64.7%).

CONCLUSION

In this study, sixty soybean genotypes were evaluated on the basis of biochemical as well as antioxidant enzymes under drought conditions. The findings suggest that the genotypes RVS-2011-77, RVS-2011-75, NRC-7, SL-96, NRC-136, AMS100-39, SL-96, RVS 2012-01, RVS-2011-73 and JS97-52 can be used for further crop improvement programme.

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