International Journal of Agricultural Technology 2021Vol. 17(4):1425-1446
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Changes in biochemical and antioxidant enzymes activities play significant role in drought tolerance in soybean

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Mishra, N., Tripathi, M. K., Tripathi, N. Tiwari, S., Gupta, N., Sharma, A. and Shrivastav, M. K. (2021). Changes in biochemical and antioxidant enzymes activities play significant role in drought tolerance in soybean. International Journal of Agricultural Technology 17(4):1425-1446.

Abstract Soybean genotypes were grouped into two clusters on the basis of biochemical profiling, anti-oxidant enzyme activities and protein profiling. Biochemical and antioxidant enzyme activity analysis among 53 genotypes revealed the presence of drought tolerance traits in three genotypes *viz*., JS97-52, RVS-14 and JS95-60. The result obtained may contribute towards improvement of soybean genotypes with the development of drought tolerant varieties with the applications of conventional as well as molecular breeding approaches. These findings also provided a base for further research to investigate the drought tolerance mechanism in soybean crop using advanced biotechnological tools.

Keywords: Antioxidant enzymes, Biochemical parameters, Drought, Protein profiling, Sustainable agriculture

Introduction

Changes in environmental conditions are responsible for evolution of new challenges. Among these, climate change is an important issue which considerably affects agriculture. To achieve the target of grain production there is a big demand of water for irrigation of crops in most of the parts of the world. Due to this drought will be a big challenge in coming days for survival of more than 40% of people belongs to 54 countries (Gardner-Outlaw and Engelman, 1997). A crop species or genotype that is pliant to low rainfall intensity and unpredictable distribution and elevated temperature would have a decisive significance for sustainable food supply to the enduringly increasing world population. To bread a cultivar for such suboptimal environment by

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means of cautiously crafted biotechnological strategies is expected to sustain food security in inhospitable climates.

Soybean is also acknowledged as a 'miracle crop' due to over 40% protein and 20% oil (Tripathi and Tiwari, 2004; Tiwari & Tripathi, 2005; Mishra *et al*., 2020; Upadhyay *et al*., 2020a; Upadhyay *et al*., 2020b). It needs an adequate water supply for the duration of its growth and development course to accomplish better yields (Buezo *et al*., 2018). The plants of soybean have been found to be affected by drought at every stage of life (Dhanda *et al*., 2004; Kachare, 2017; Kachare *et al*., 2019; Mishra *et al*., 2021a; Sharma *et al*., 2021). Significant reductions in the levels of chlorophyll a, b, and total chlorophyll have been observed due to drought in soybean crop (Wu and Zhang, 2019). Plants develop various mechanisms to fight different stresses (Specht *et al*., 2001) and these mechanisms may be due to alteration in biochemical pathways. Numerous biochemical parameters have been exploited to recognize tolerant genotype (s) to drought (Wang *et al*., 2019; Sahu *et al*., 2020; Choudhary *et al*., 2021).

To distinguish the desired genotype(s), various biochemical parameters that are being utilized for selection are proline, membrane stability, total sugar, MDA, protein, antioxidant activities (catalase, glutathione reductase and peroxidase) etc. Earlier, it is reported that the antioxidant enzymes play major role in control, and removal of reactive oxygen species (ROS). The increased activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been observed in crops as well as soybean under water stress (Kachare, 2017; Kachare *et al*., 2019; Wang *et al*., 2019). Enhancement of MDA content has also been recorded under water deficit in crops (Wang *et al*., 2019; Sahu *et al*., 2020).

Identification of drought tolerant genotypes due to biochemical alterations may provide a basis for the development of new plant varieties using conventional (Mishra *et al*., 2021b) as well as molecular breeding approaches to fight water stress. The current investigation was executed to monitor drought tolerant soybean genotypes based on manifestation of different biochemical parameters, antioxidant enzymes activities and protein profile.

Materials and methods

The present investigation was consisted of 53 soybean genotypes (Table 1) with diverse reactions to drought *viz*., susceptible and tolerant. The seeds were obtained from College of Agriculture, JNKVV, Jabalpur, RAK, College, Sehore and Zonal Agricultural Research Station, Morena, Madhya Pradesh, India.

Table 1. List of soybean genotypes with their parentage

S. No.	Genotypes	Source/Pedigree	S. No.	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	**28.**	RSC-10-52	NRC 37X JS335
2.	JS 20-69	JS 97-52 x SL 710	**29.**	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	**30.**	SL-1068	SL755XSL525
4.	JS 20-98	JS 97-52x JS SL710	**31.**	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	**32.**	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	**33.**	MACS725	JS93-05X MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	**34.**	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	**35.**	NRC -125	EC54688xps1044
9.	JS 97-52	PK 327 x L 129	**36.**	NRC-132	JS97-52X PI086023
10.	JS 20-84	JS 98-63 x PK 768	**37.**	NRC-134	NRC7XAGS191
11.	JS 20-34	JS 98-63 x PK 768	**38.**	NRC SL-1	JS335XSL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	**39.**	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	**40.**	PS 1613	PS1225XPS1042
14.	RVS 2011-35	JS 335 X PK 1042	**41.**	AMS 2014-1	AMS99-33XH6P5
15.	RVS 2001-4	JS 93-01x EC 390981	**42.**	KDS 992	JS93-05XEC241780
16.	RVS -14	JS 93-05x EC 390981	**43.**	VLS -94	VL Soya59X VS2005-1
17.	RVS -24	J.P 120 x JS 335	**44.**	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110XJSM66	**45.**	RVS 76	MAUS-162XJSM-66
19.	NRC- 76	NRC-37XL-27	**46.**	NRC127	JS97-52XPI542044
20.	NRC -86	RKS15XEC481309	**47.**	KDS980	JS93-05XAMS1
21.	NRC- 130	EC390977XEC538828	**48.**	G-29	Germplasm
22.	NRC -131	EC390977XEC538828	**49.**	RSC-10-70	JS335X Bragg
23.	NRC -147	Germplasm accessions C210	**50.**	RSC-10-71	Bragg XJS335
24.	AMSMBC -18	Mutant of Bragg	**51.**	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	**52.**	MACS-15-20	NRC37XMohetta
26.	MACS – 1520	EC241780XMACS330	**53.**	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044XJS9305			

The field experiment was conducted at the experimental field and the laboratory work at Biochemical Analysis Laboratoray, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madaya Pradesh, India during *Kharif* 2018-19. The investigational region covered was fairly identical in term of topography and fertility. Gwalior has subtropical, semi-arid climate and chilly winters with random showers. The average rainfall was about 312.0 mm in July, 190.6 mm in August, 166.4 mm in September and 0.0 mm in the month of October respectively. Crop was shown on July 2019. Between 60^{th} to 70^{th} days of crop growth season neither rain has been received nor has irrigation given manually. Leaves were collected after 70 days of sowing from five random selected plants of each line for the analysis of diverse biochemical parameters. Completely Randomized Design (CRD) was adopted and the data were analyzed as per method suggested by Snedecor and Cochran (1967).

Biochemical analysis

Photosynthetic pigments (chlorophyll a, b and total) were quantified using UV-VIS spectrophotometer at 470, 645 and 663 nm absorbance and calculated according to Arnon's equation (1949). Proline content in leaves was determined as suggested by Bates *et al.* (1973). Estimation of sugar content (mgg^{-1} fresh weight) was estimated as methods adopted by Kachare (2017) in soybean. Malondialdehyde test (Lipid peroxidation assay, nmol $g^{-1}FW$) estimation was exercised with the help of method developed by Stewart and Bewley (1980). Membrane stability index (MSI) was worked out as formulae suggested by Razzaq *et al.* (2013). Total seed protein content was estimated by the process of Bradford (1976).

Determination of antioxidant enzyme activity (catalase, glutathione reductase and peroxidase)

Sample preparation was done according to the method adopted by Kachare (2017). Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was computed as per method of Nakano and Asada (1981). Catalase (CAT) activity (EC 1.11.1.6) was analyzed by the UV process as proposed by Aebi (1983). Glutathione reductase (GR) activity (EC 1.6.4.2) was estimated by the technique as explained by Smith *et al*. (1988). Guaiacol peroxidase (PDX)

activity (EC 1.11.1.7) was computed by estimating the oxidation of guaiacol as protocol described by Rao *et al*. (1996).

Protein's characterization

The extracted protein was used to analyze through SDS-PAGE method according to the steps suggested by Laemmli (2011).

Results

Biochemical parameters

Variations among 53 soybean genotypes for studied biochemical traits with analysis of variance are shown in Table 2 which indicated that the alteration in their response against drought stress. Total Chlorophyll content in $mgml^{-1}$varied considerably in ranged of 38.13-59.87 $mgml^{-1}$, with maximum in genotype JS 97-52 (59.87 $mgml^{-1}$) chased by genotypes: RVS-14 (56.29 $mgml^{-1}$), JS20-94 (55.88 $mgml^{-1}$) and NRC-76 (55.07 $mgml^{-1}$). Whereas the minimum was documented in genotype JS20-84 (38.13 $mgml^{-1}$) pursued by genotypes RVS2011-35 (40.01 mgml-1), NRC-130 (41.21 $mgml^{-1}$) and MACS-1520 (41.58 $mgml^{-1}$). Proline content varied significantly in ranged of 66.25-111.40 μgg^{-1} with highest in genotype JS 97-52 (111.40 μgg^{-1}), tracked by genotypes RVS-14 (103.45 μgg^{-1}), JS 95-60 (93.95 μgg^{-1}) and RVS 2001-4 (91.54 μgg^{-1}). However, the least showed in genotype JS335 (66.25 μgg^{-1}) followed by genotypes: JS 20-29 (67.90 μgg^{-1}), NRC-76 (68.05 μgg^{-1}), NRC-134 (68.15 μgg^{-1}) and SL-1123 (68.30 μgg^{-1}). Total Sugar in mgg^{-1}varied significantly between 2.35-5.45 mgg^{-1} with utmost in genotype JS97-52 (5.45 mgg^{-1}) which is pursued by genotypes RVS-14 (5.15 mgg^{-1}) and JS95-60 (5.15 mgg^{-1}) while lowest in genotype KDS992 (2.35 mgg^{-1}), tracked by genotype RSC-10-71 (2.45mgg^{-1}). MDA content in nmole gm^{-1}differed significantly in ranged of 42.65 to 60.80 with greatest in genotype JS 97-52 (60.80 nmole gm^{-1}) tracked by JS95-60 (57.22 nmole gm^{-1}) and least amount (42.65 n mole gm^{-1}) in genotype RVS2007-6. Membrane stability is one of the major components in tolerance under stress. Highest membrane stability (MS) was texted in genotype JS97-52 (64.50 %) intimately trailed by genotypes RVS-14 (63.00%) and JS95-60 (61.00%), while minimum (29%) in genotype RSC-10-70. Protein synthesized in immature seeds varied significantly among 53 soybean genotypes in ranged of 34.4%-39.3% with upmost in genotype JS20-71 (39.30

%) closely pursued by genotypes: AMS-100-39 (39.10%) and MACS725 (38.6%%). Whilist the lowest amount was documented in genotype JS95-60 (34.4%) tracked by genotypes JS 20-69 (34.5%) and SP 37 (34.8%).

Biochemical parameters based hierarchical cluster analysis

The hierarchical cluster analysis and the content values are presented in Table 2, and the dynamic expression profile was determined and is shown in Figure 1. Multivariate analysis based on diversity was performed using the UPGMA. The mean value of biochemical parameters of different genotypes falling in each cluster was presented in the generated dendrogram for distinguished into two major clusters *i.e.,* I and II. Cluster I divided into subclusters. These clusters further subdivided into minor clusters. Cluster I consisted genotypes RVS-2001-4 and KDS-980 as an out group and cluster II also consisted two genotypes *viz,* JS-97-52 and RVS-14 as an outgroup. The biochemical parameters illustrated similar pattern in both of these two genotypes, and they were clustered together.

Principal component analysis (PCA) of biochemical parameters

Principal component analysis (PCA) was done by considering biochemical variables simultaneously. The pattern of variations illustrated by the PCA described by correlation coefficients determined for pair-wise association of the traits. Genotypes JS-97-52 and RVS-14 situated at the unique position of the plot. The PCA correlation depicted those genotypes possessed higher and lower magnitudes of biochemical parameters occupying unique position towards the graph (Figure 2).

Activities of antioxidant enzymes

The analysis of variance (Table 2 and Figure 3) clearly indicated presence of ample variations among 53 soybean genotypes for all antioxidant enzymes activities. APX was found to be maximum in genotype JS97-52 (2.21-unit mg^{-1} protein min^{-1}) whereas minimum (0.23-unit mg^{-1} protein min^{-1}) was documented in genotype JS20-94. Catalase activity was found to be highest (0.97-unit mg^{-1} protein min^{-1}) in genotype JS 97-52 while lowest (0.30-unit mg^{-1} protein min^{-1}) was observed in genotype NRC-132. Highest Gluthione reductase (GR) activity was texted in genotype JS 97-52 (0.87-unit mg^{-1}protein

min^{-1}) narrowly pursued by genotypes: JS 95-60 (0.80 unit mg^{-1}protein min^{-1}), however, least in RVS-18 (0.22 unit mg^{-1}protein min^{-1}). Guaiacol peroxidase (PDX) ranged between 0.24-1.91 unit mg^{-1} protein min^{-1} with greatest (1.91 unit mg^{-1} protein min^{-1}) in genotype JS 97-52 and lowest (0.24 unit mg^{-1} protein min^{-1}) in genotype JS20-98. Further, elevated PDX activity was examined in genotypes *i.e.*, JS 97-52, RVS 2001-4, NRC-76 and RVS-14.

Antioxidant activities based cluster analysis

In antioxidant enzymes data based dendrogram, the genotypes were separated into two clusters *i.e.,* major and minor cluster. The major cluster contained 49 genotypes while the minor cluster had only 4 genotypes *namely*: RVS-24, JS20-84, RVS-14 and JS97-52. The major cluster was further divided into two groups. The major group is consisted 47 genotypes while minor group had only two genotypes *viz.,* NRC-76 and RVS2001-4. The major group was further divided into two sub-groups. Major sub-group contained 38 genotypes and minor sub-group consisted nine genotypes including AMS2014-1, PS-1613, VLS-94, PS-10-92, RSC-10-70, NRC-SL-1, NRC-134, NRC-132 and NRC-125. Among these nine genotypes utmost resemblance was documented between AMS2014-1 and PS-1613 and both genotypes grouped together (Figure 4).

Protein profiling through SDS-PAGE

In this experimentation, we analyzed immature seed protein pattern for fifty-three soybean genotypes using SDS-PAGE (Figure 5). Dendrogram was generated on the basis of banding pattern and studied genotypes were divided into two clusters (Figure 6). The major cluster is consisted 50 genotypes while minor cluster had only three genotypes, *viz.*, SL-1068, AGS-111 and NRC-132. Among these three genotypes, NRC-132 was found to be diversed and grouped distantly from rest of the two genotypes. The data were also analyzed on the basis of band intensity. The variations in the intensity of bands with similar molecular weight indicated the expression capability of genotypes of same protein in different genotypes.

Table 2. Mean performance of different biochemical parameters and anti-oxidant enzymatic activities of soybean genotypes

S. No.	Parameter Genotypes	Total Chlorophyll Content (mg/ml)	Proline (µg/g)	Total Sugar (mg/g)	MDA (n mole/gm)	MSI (%)	Protein (%)	Ascorbate peroxidase (unit/mg protein/min)	Catalase (unit/mg protein/min)	Glutathione reductase (unit/mg protein/min	Guaiacol peroxidase (unit/mg protein/min)
1.	JS 20-29	53.68	67.90	3.15	46.70	50.50	36.2	0.57	0.74	0.43	0.32
2.	JS 20-69	49.90	70.35	2.70	43.57	45.00	34.5	0.40	0.72	0.30	0.51
3.	JS 335	52.30	66.25	2.75	45.39	50.5	35.6	0.58	0.40	0.29	0.62
4.	JS 20-98	52.79	71.45	3.00	47.35	37.00	37.2	0.67	0.53	0.36	0.24
5.	JS 20-94	55.88	90.40	3.90	45.17	40.50	38.1	0.23	0.44	0.41	0.42
6.	JS 93-05	50.06	75.65	3.00	44.67	36.50	37.4	0.45	0.50	0.27	0.65
7.	JS 20-116	52.39	76.05	3.75	42.73	48.00	36.1	0.31	0.64	0.27	0.75
8.	JS 95-60	47.55	93.95	5.15	57.22	61.00	34.4	0.27	0.43	0.80	0.60
9.	JS 97-52	59.87	111.4	5.45	60.80	64.50	35.7	2.21	0.97	0.87	1.91
10.	JS 20-84	38.13	81.05	4.55	46.19	49.50	36.8	1.79	0.80	0.30	0.72
11.	JS 20-34	47.13	70.50	4.30	47.07	40.50	37.1	0.26	0.85	0.34	0.39
12.	JS 20-71	50.25	77.80	3.75	46.66	39.50	39.3	0.29	0.57	0.52	0.45
13.	RVS 2007-6	47.48	70.95	2.80	42.65	53.50	37.2	0.33	0.44	0.26	0.71
14.	RVS 2011-35	40.01	81.35	3.05	45.88	43.50	38.2	0.46	0.47	0.32	0.50
15.	RVS 2001-4	41.74	91.50	2.75	47.78	45.00	36.8	0.59	0.43	0.76	1.65
16.	RVS -14	56.29	103.45	5.15	52.93	63.00	38.1	1.70	0.86	0.80	1.24
17.	RVS -24	50.39	77.65	3.00	55.12	38.50	36.2	2.04	0.64	0.27	0.37
18.	RVS -18	47.10	79.85	3.30	53.67	46.50	35.7	0.50	0.72	0.22	0.45
19.	NRC- 76	55.07	68.05	4.65	46.87	35.50	37.1	0.32	0.96	0.77	1.58
20.	NRC -86	47.20	82.00	2.65	48.20	44.50	37.8	0.47	0.63	0.63	0.57

Table 2 (Con.)

S. No.	Parameter Genotypes	Total Chlorophyll Content (mg/ml)	Proline (µg/g)	Total Sugar (mg/g)	MDA (n mole/g m)	MSI (%)	Protein (%)	Ascorbate peroxidase (unit/mg protein/min)	Catalase (unit/mg protein/min)	Glutathione reductase (unit/mg protein/min	Guaiacol peroxidase (unit/mg protein/min)
21.	NRC- 130	41.21	78.95	2.60	45.29	39.50	36.5	0.48	0.64	0.60	0.59
22.	NRC -131	52.46	87.30	3.95	43.20	51.00	37.2	0.40	0.52	0.52	0.40
23.	NRC -147	46.95	87.90	3.70	53.93	36.50	36.2	0.30	0.55	0.46	0.78
24.	AMSMBC -18	47.81	81.25	3.80	55.08	48.00	38.1	0.26	0.76	0.36	0.32
25.	AMS-100-39	49.09	80.50	3.75	46.61	44.50	39.1	0.24	0.40	0.63	0.51
26.	MACS – 1520	41.58	70.95	3.15	45.76	55.00	37.4	0.55	0.61	0.58	0.79
27.	MACSNRC-1575	48.02	86.80	3.50	45.62	57.50	37.9	0.43	0.55	0.49	0.57
28.	RSC-10-52	46.07	70.15	3.05	50.20	45.00	38.1	0.41	0.35	0.40	0.40
29.	SL -1123	44.20	68.30	2.65	52.71	32.50	36.2	0.34	0.64	0.39	0.61
30.	SL-1068	43.08	91.50	4.20	54.80	53.00	35.7	0.28	0.37	0.32	0.57
31.	AGS 111	46.63	87.80	4.75	50.58	49.00	37.4	0.42	0.46	0.52	0.85
32.	EC457286	49.68	83.45	4.25	54.56	31.00	37.1	0.38	0.44	0.55	0.71
33.	MACS725	51.59	88.75	3.90	45.41	31.00	38.6	0.32	0.50	0.45	0.90
34.	SP 37	48.51	81.30	3.55	46.09	41.00	34.8	0.43	0.69	0.40	0.53
35.	NRC -125	51.42	85.60	4.05	50.55	50.50	37.6	0.87	0.43	0.46	0.63
36.	NRC-132	47.08	85.75	3.25	52.92	57.00	37.9	0.77	0.30	0.62	0.60
37.	NRC-134	52.14	68.15	3.70	45.33	32.50	37.1	0.82	0.36	0.60	0.64
38.	NRC SL-1	41.69	82.30	2.90	51.42	40.50	38.1	0.80	0.63	0.60	0.63
39.	PS 1092	46.87	90.85	2.60	46.63	52.00	37.2	0.82	0.39	0.24	0.81
40.	PS 1613	49.63	78.30	3.00	43.53	51.00	38.4	0.70	0.44	0.49	0.87

Table 2 (Con.)

S. No.	Parameter Genotypes	Total Chlorophyll Content (mg/ml)	Proline (µg/g)	Total Sugar (mg/g)	MDA (n mole/g m)	MSI (%)	Protein (%)	Ascorbate peroxidase (unit/mg protein/min)	Catalase (unit/mg protein/min)	Glutathione reductase (unit/mg protein/min	Guaiacol peroxidase (unit/mg protein/min)
41.	AMS 2014-1	45.19	80.00	2.75	45.60	51.50	37.2	0.76	0.43	0.47	0.92
42.	KDS 992	50.18	70.35	2.35	45.13	34.50	36.5	0.44	0.59	0.34	0.85
43.	VLS -94	48.38	82.00	3.05	50.68	37.00	36.2	0.63	0.39	0.25	0.80
44.	SKF-SPS -11	40.42	85.30	3.35	53.92	51.00	37.4	0.51	0.43	0.31	0.73
45.	RVS 76	42.42	71.65	2.95	54.92	51.00	36.8	0.68	0.52	0.36	0.58
46.	NRC127	48.76	70.65	2.50	54.91	52.00	37.1	0.44	0.50	0.39	0.46
47.	KDS980	42.19	71.45	2.95	55.23	41.50	35.7	0.47	0.57	0.48	0.72
48.	G-29	44.14	78.55	2.60	55.10	47.50	36.2	0.40	0.45	0.36	0.55
49.	RSC-10-70	46.64	76.00	3.00	54.78	29.00	37.1	0.80	0.64	0.54	0.56
50.	RSC-10-71	51.00	71.55	2.45	44.11	50.50	36.4	0.52	0.60	0.59	0.73
51.	NRC-2	45.78	75.55	2.80	56.41	50.50	37.2	0.67	0.42	0.32	0.60
52.	MACS-15-20	50.98	77.70	2.70	56.37	58.50	37.9	0.62	0.49	0.36	0.47
53.	MACS-58	51.90	81.05	2.85	45.21	48.00	36.5	0.45	0.33	0.45	0.33
SE (m)		**1.076566**	**1.130809**	**0.154004**	**3.160806**	**0.845032**	**0.147046**	**0.009329**	**0.017895**	**0.014916**	**0.016474**
$CD_{0.05}$		**3.057168**	**3.211204**	**0.437332**	**8.97587**	**2.399671**	**0.417574**	**0.026**	**0.050817**	**0.042358**	**0.046783**
Range		**38.13-59.87**	**66.25-111.40**	**2.35-5.45**	**42.65-60.80**	**29.00-64.50**	**34.4-39.3**	**0.23 - 2.21**	**0.30-0.97**	**0.22-0.87**	**0.24-1.91**

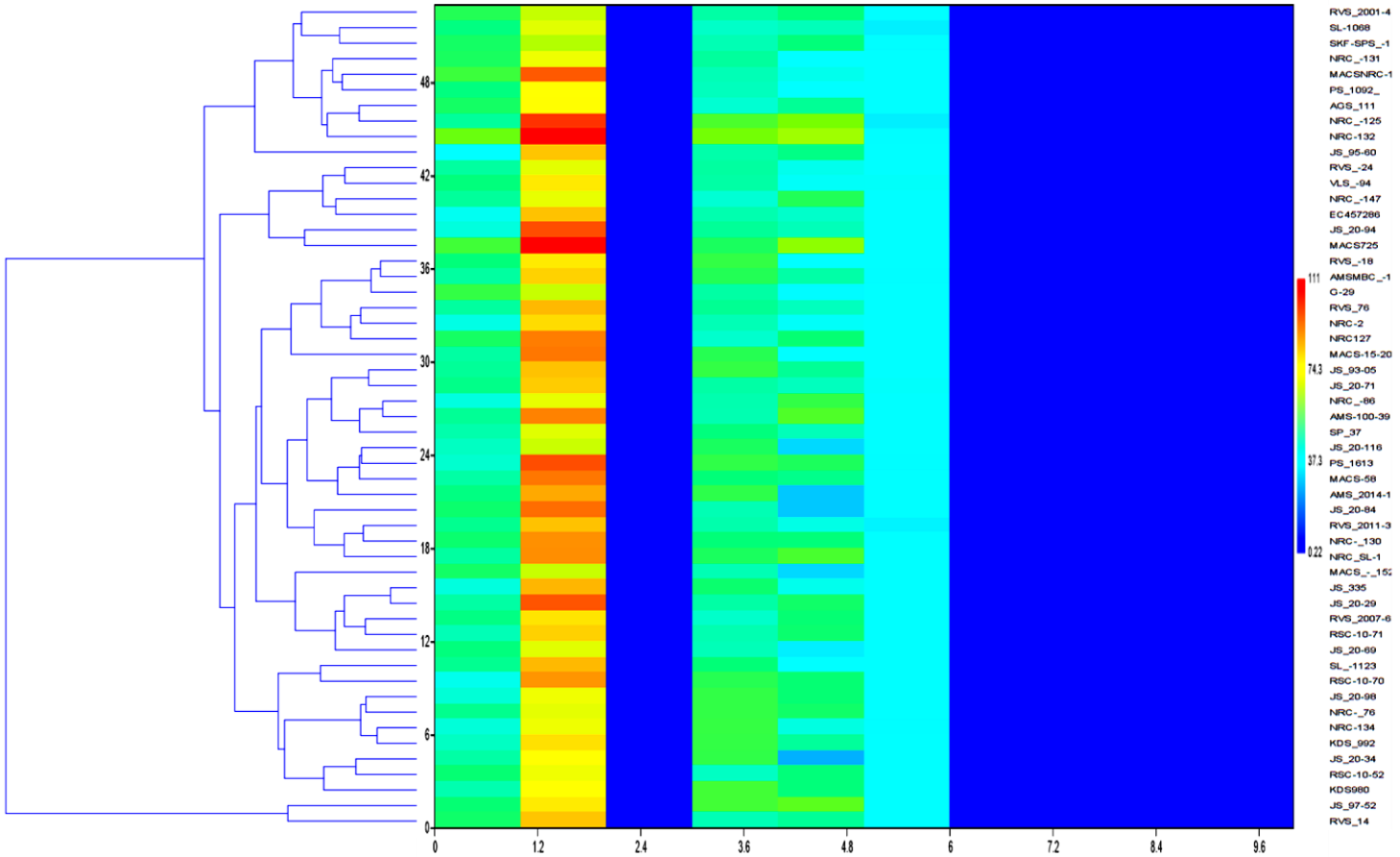

Figure 1. Biochemical based hierarchical cluster analysis of soybean genotypes based on different biochemical parameters (total chlorophyll content, proline, total sugar, MDA, MSI and protein %)

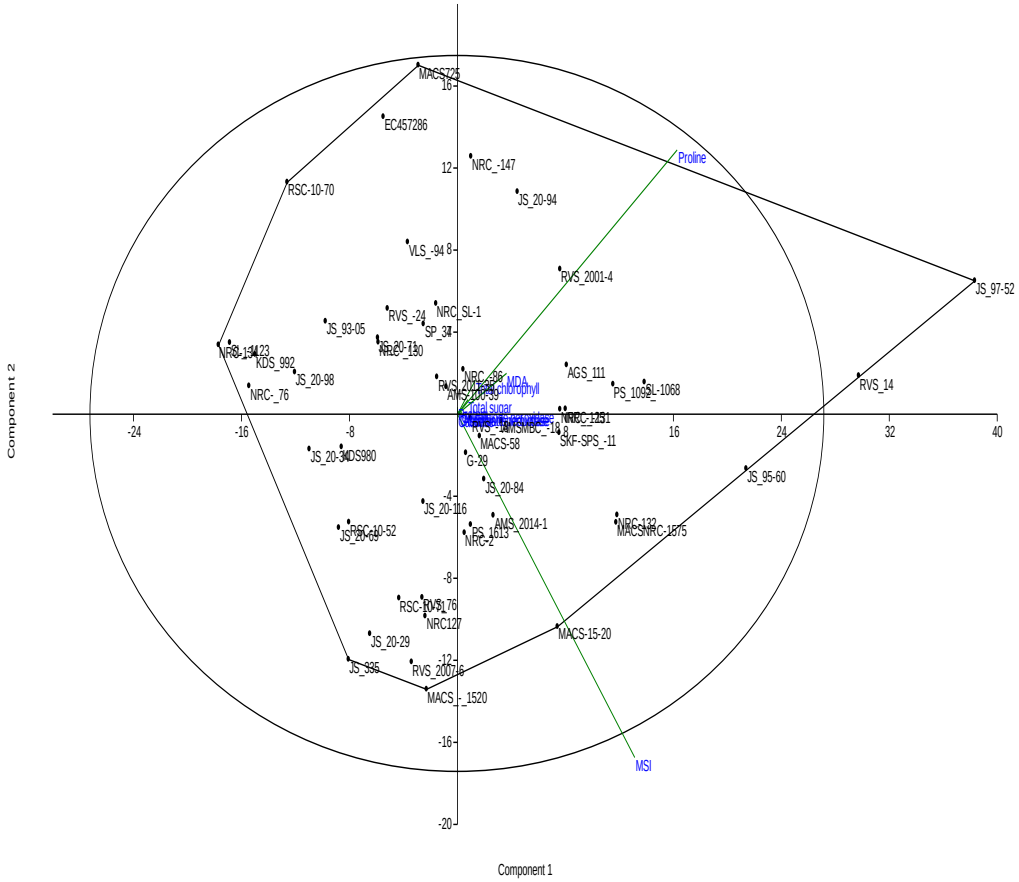

Figure 2. Principal Component analysis based on different biochemical showing relationship among soybean genotypes

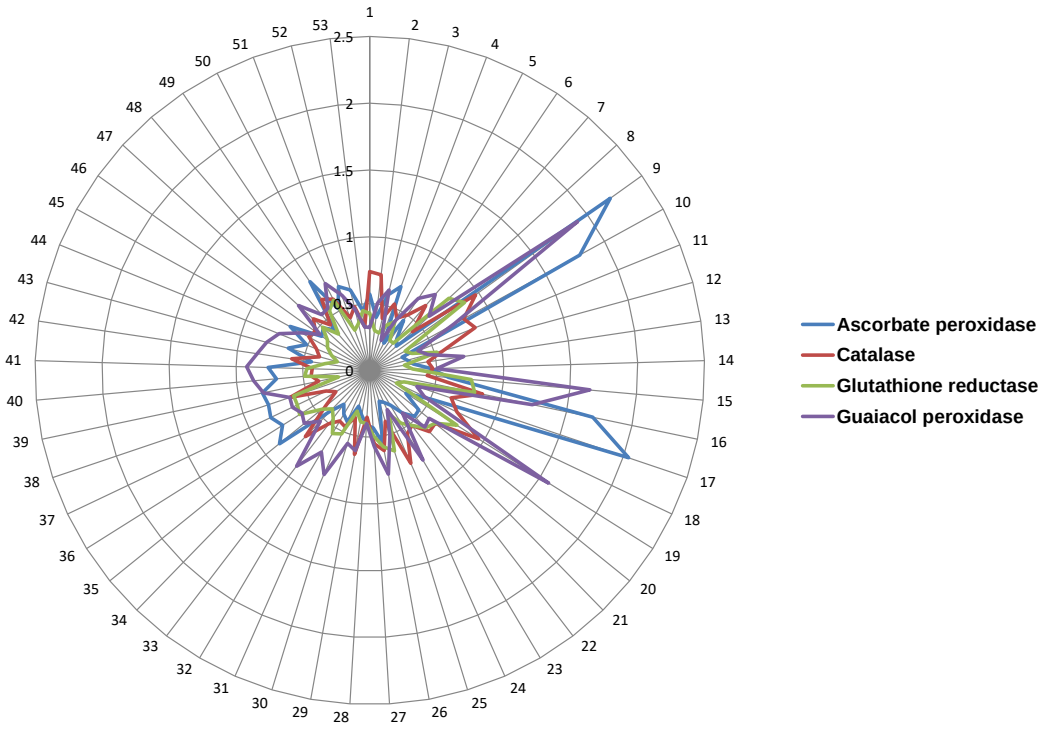

Figure 3. Anti-oxidative enzymes activities of different genotypes

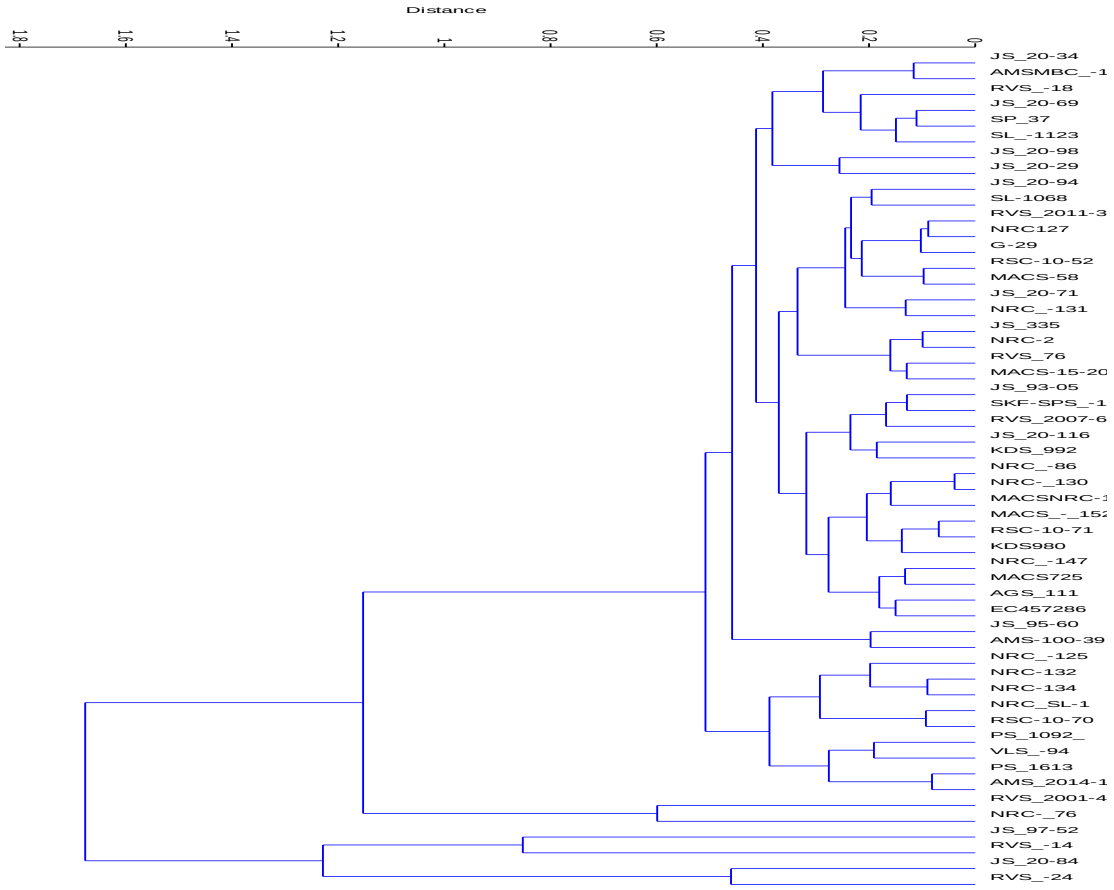

Figure 4. Dendrogram showing relationship among soybean genotypes based on different antioxidative enzymes (Ascorbate peroxidase, Catalase, Glutathione reductase and Guaiacol peroxidase)

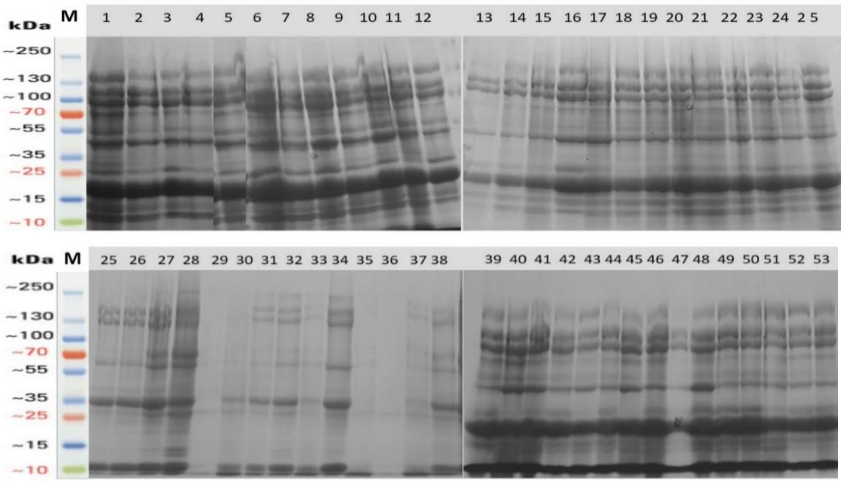

Figure 5. SDS protein profiling of 53 soybean genotypes

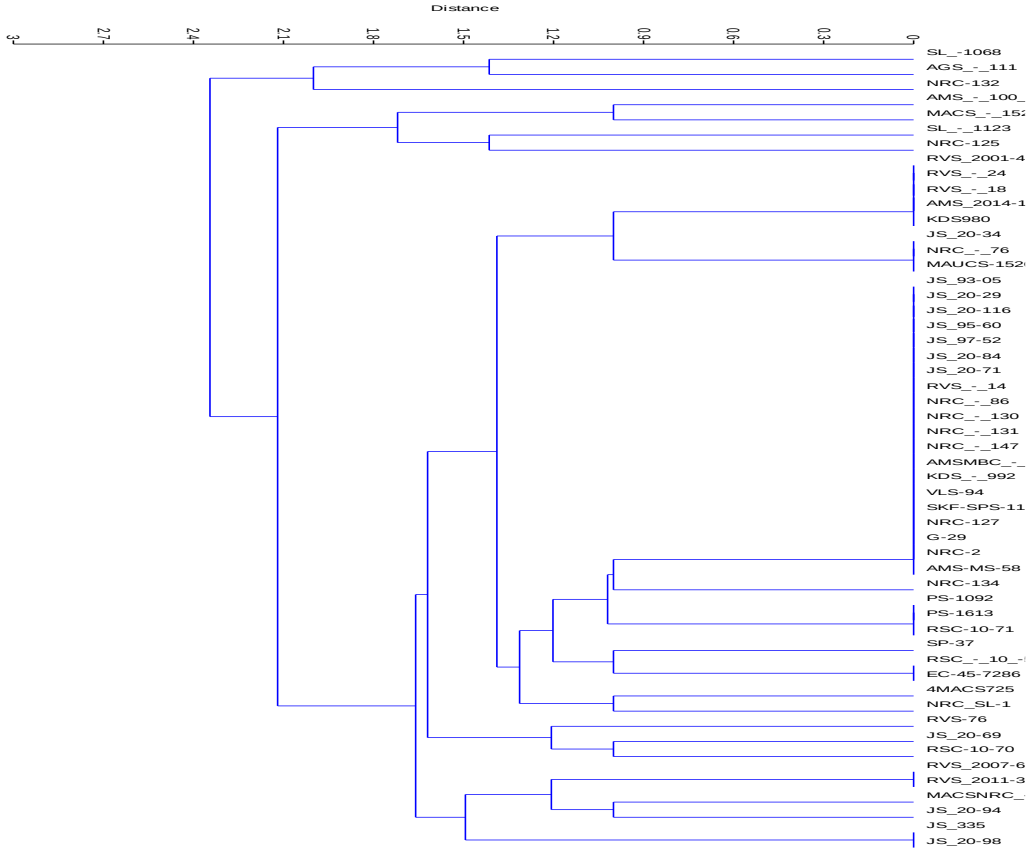

Figure 6. Dendrogram showing relationship among different soybean genotypes based on SDS- Profiling

Discussion

Biochemical parameters

Generally, the level of chlorophyll content in leaves determines the rate of photosynthesis. In the current study, a reduction in chlorophyll component was found in susceptible genotypes in comparison to tolerant genotypes. Similar results in soybean were found earlier by Hossain *et al*. (2015). Reduced level of chlorophyll synthesis in susceptiable genotypes may be the reason of less activity of the photosynthetic elements. Previously, loss of chloroplast membranes under drought stress has also been documented by Anjum *et al*. (2011). Parallel reduction in chlorophyll levels in several other plant species *viz*., soybean, maize, rice, chickpea, peal millet *etc.* have been reported (Zhang *et al*., 2007; Sahu *et al*., 2020; Choudhary *et al*., 2021; Sharma *et al*., 2021). High reduction in chlorophyll content was found in drought susceptible genotypes in the present study.

Role of proline in osmotic regulation under water stress has been monitored in various plant species (Rengasamy, 2002; Guo *et al*., 2009). Soybean genotypes with significant rise in proline contents have been considered as drought tolerant. Highest proline content increment was found in genotype JS97-52 followed by genotypes RVS2001-4 and JS95-60. Previously, Khan *et al*. (2015), Kachare *et al*. (2019) and Sharma *et al*. (2021) observed comparable trend in the proline content during their study on screening of soybean genotypes tolerant to drought stress. Increased proline content maintains cell water level under drought (Ghorbanli *et al*., 2012; Choudhary *et al*., 2021). Further, George *et al*. (2015) suggested that increased proline has osmoprotective functions by preventing separation of enzymes during metabolic activities.

Among osmotic regulating substances, total soluble sugars are noteworthy contributors of drought tolerance (Gurrieri *et al*., 2020; Choudhary *et al*., 2021). During present investigation, genotype JS97-52 had maximum soluble sugar tracked by genotypes RVS2001-4 and JS95-60. These findings indicate the presence of possible drought tolerance in these genotypes. Previously, Kachare *et al*. (2019) and Sharma *et al*. (2021) also observed momentous enhancement in total soluble sugar content under drought stress while studying on Indian soybean genotypes.

Lipid peroxidation mechanism is a cursor of oxidative stress under water stress. Malondialdehyde (MDA) content is considered as a sign of membrane lipid peroxidation and shows the level of damage in membrane underneath stress (Wang *et al*., 2019). MDA content in nmole $gm^{-1}$was found

greatest in genotype JS 97-52. Genotypes exhibited higher MDA content in leaves might be tolerant against drought (Tatar and Gevrek, 2008) due to more synthesis of Reactive Oxygen Species in comparison of the rest of the genotypes and this parameter can be applicable to find out drought tolerance in genotypes (Chug *et al*., 2011).

According to Blackman *et al*. (1995) the increments in MSI indicates the reduction of lipid peroxidation with oxidative bursts under water stress conditions. In previous studies, MSI has been used frequently for screening of drought tolerance in crop species (Farooq and Azam, 2002). In similar studies, Almeselmani *et al*. (2012), Kachare *et al*. (2019) and Sharma *et al*. (2021) reported significant genotypic differences in membrane stability as an indirect criterion for selection of drought tolerance in soybean.

Anti-oxidant enzymes

Anti-oxidative enzymes have vital role in the protection of plants in water stress due to their contribution in tolerance mechanism (Blokhina *et al*., 2003; Xue *et al*., 2011). This may be entire capacity of a particular genotype to combat against these stresses. Previous reports proved the role of antioxidant enzymes in the mechanism of drought and dehydration tolerance in soybean (Vasconcelos *et al*., 2009; Xue *et al*., 2011). Major system in plants under abiotic stress for detoxification of hydrogen peroxide is the ascorbate-glutathione cycle in which Ascorbate peroxidase (APX) helps in the conversion of H_2O_2 into H_2O, in chloroplast (Mittler and Zilinskas, 1994; Correa-Aragunde *et al*., 2013). Enhanced APX activity was invented in genotypes JS97-52 followed by RV-24 as compared to rest of the genotypes possibly due to appearance of prominent H_2O_2 detoxification with prevention of H_2O_2-mediated cell damage (Kommavarapu *et al*., 2013). Increased APX activity in soybean under drought stress has been earlier reported by Kausar *et al*. (2012), Kachare *et al*. (2019) and Sharma *et al*. (2021). Catalase (CAT) enzymes are capable to alter millions of H_2O_2 molecules into H_2O and O_2 in a second (Chelikani *et al*., 2004). Enhanced CAT and GR activity was found in soybean genotype JS97-52. In an earlier research, conducted by Masoumi *et al*. (2011) a positive correlation between drought tolerance in plant genotypes and enhanced CAT was reported. Similarily, Liu *et al*. (2013), Kachare *et al*. (2019) and Sharma *et al*. (2021). also observed a significant increase in GR activity in some soybean genotypes. Earlier, Porcel *et al*. (2003) reported increased GR activity in drought tolerant soybean genotypes. This indicates the enhanced ROS scavenging ability of drought tolerant plants as a result negligible injure to plants under stress. Role of GR as an important enzyme to sustain redox

condition of plant by converting oxidized glutathione (GSSG) into reduced glutathione (GSH) with the help of NADPH has also been proved by Garg *et al*. (2012). Enhanced PDX activity was evidenced in genotype JS97-52 while lowest was in genotype JS20-98. This indicates the possible tolerance in these genotypes as earlier stated by Murthy *et al*. (2012). Similar to the present research, Kachare (2017) and Sharma *et al*. (2021) also noticed directly proportional relation between PDX activity and level of water stress in soybean. Akitha-Devi *et al*. (2015) reported enhanced activities of antioxidant enzymes with reduction in osmotic potential. These findings clarify the relation between activity of antioxidant enzyme and defense mechanisms against water stress in soybean.

Protein profiling

Soybean seed contains about 35-40% proteins with big contribution of essential amino acids. It is also a rich source of antioxidants and unsaturated fatty acids. Many of these proteins have functions in the management of water deficit (Qayyum *et al*., 2011). These proteins play major role in uptake of water from environment for the betterment of plant health. So, the proteins help the plants in completion of growth and development cycle normally. On average, 12 bands per genotype were detected in a range of molecular weight from 3.5 to 43.0 kDa in present study correspondingly to Arumingtyas and Savitri (2014) and Kachare (2017). Sahu *et al*. (2020) and Gupta *et al*. (2021) also detected range of protein bands in chikpea.

It is concluded that the basis of different biochemical parameters and anti-oxidant enzymes activities genotypes *viz.*, JS97-52, RVS-14 and JS95-60 were found with drought tolerance. The findings of the present study provide a base for the use of these soybean genotypes for further hybridization to develop drought tolerant varieties. These results also open the door for the applications of advanced biotechnological tools for deep analysis of drought tolerance mechanism in soybean crop.

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(Received: 4 May 2021, accepted: 30 June 2021)