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Identifying Drought Tolerant Genotypes in Okra with Physiological and Molecular Approaches

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***Abstract*:** Okra (*Abelomoschus esculentus* L.) is an annual herbaceous plant and belongs to family Malvaceae. Drought stress affects okra growth and productivity, disrupting physiological functions and the photosynthetic rate, resulting in yield losses. By understanding the physiological response of okra genotypes under drought stress conditions is critical to the selection of drought-tolerant accessions. Hence, to identify drought tolerance lines, initially selected thirty genotypes for preliminary drought screening, later advanced with six relatively promising genotypes *viz.* P-8, White velvet, Arka abhay, Parbani kranti and Arka anamika and Bagalkot local were considered for further study. The plants were exposed different water potential levels stresses by induced slanting glass plate technique in the laboratory. The genotypes performance was analysed at different Poly ethylene glycol-6000 osmotic stress concentrations for germination, RWC, SPAD value, and root traits up to 22 days at seedling stage revealed Arka abhay and Parbani kranti lines as relatively drought tolerant. These genotypes further exposed to water stress (65% Field capacity) with control under field evaluation. The genotypes Arka abhay and Parbani kranti were drought tolerant genotypes with higher RWC, root length, lateral roots, chlorophyll and pod yield under induced stress conditions. The genotypes were analyzed for genetic diversity with ISSR DNA molecular markers. The dendrogram revealed tolerant lines were grouped in one cluster and the rest in other clusters. Arka abhay and Parbani kranti were relatively drought tolerant and could be suitable to grow under mild water stress condition.

***Keywords*:** Chlorophyll, Drought, ISSR, Okra, PEG-6000, Roots, SLW, Water potential

Okra is a commercial vegetable crop belongs to family Malvaceae which can be grown on wide range of soils, but well drained fertile soils with adequate organic matter result to high yield (Akinyele and Temikotan 2007). The crop is widely cultivated throughout the year in the tropics. Drought is one of the major ecological factors limiting crop production and food quality globally, especially in the arid and semi-arid areas of the world. Drought alone accounts for yield losses ranging between 30 and 100% in okra, primarily when the stress occurs during the flowering and pod-filling stages (Mkhabela et al 2021). Okra plant plants subjected to a low level of stress (watered once a week) performed better than those moderately stressed. Water stress is one of the limiting factors in crop growth and yield which reduces dry matter production, yield and yield components through decreasing leaf area and accelerating leaf senescence (Emam and Seghatoleslami 2005). One of the most common methods used to determine the tolerance of plants to abiotic stresses is the evaluation of the germination capacity of seeds under abiotic stress conditions (Larcher 2000). Hence, in this direction conducted a study with an objective of screening of the okra genotypes by physiological drought tolerant traits under both laboratory and field ultimately to find out relatively drought tolerate okra lines.

MATERIAL AND METHODS

The experiment was conducted during the years 2014-15 and 2015-16 at University of Horticultural Science, Udyanagiri, Bagalkot, Karnataka, India. It is situated at 14˚47' Northern latitude, 75˚59' East longitude and at an altitude of 612.05 meters above the mean sea level. Initially thirty okra genotypes were screened for water stress basal study. Out of which we chose six relatively promising genotypes for further extensive drought tolerance study.

Laboratory study by PEG-6000: The experiment has been laid out in factorial randomized block design with main factor (Factor 1) includes four PEG-6000 concentrations whereas, sub factor (Factor 2) includes six genotypes (P-8, White velvet, Arka Abhay, Parbhani Kranti, Arka Anamika and Bagalkot local) with three replications. The experiment was carried out in the laboratory condition under ambient temperature. To induce water stress, 0%, 5%, 10% and 15% Poly ethylene glycol 6000 (PEG-6000) concentrations solutions were used for the study. It represents the decrease in water potential as the concentration of the PEG-6000 solutions increases. The seeds were allowed to grow in above PEG-6000 solutions by slanting glass plate technique. The observations *viz.,* seed germination (%), SPAD value, shoot length(cm), root length(cm), number of lateral roots

and relative water content were recorded with 14 and 22 days after sowing.

Field evaluation by induced water stress: Under field study, six okra genotypes were grown under two water regimes conditions *viz.* well irrigated condition and water stressed condition (65% field capacity). The field capacity was maintained by controlled intervals water supply to plots. The experiment was laid out in split plot design with main factor (Factor 1) includes well irrigated and water stress condition whereas the six okra genotypes as sub factor (Factor 2) with three replications. Under induced water stress level, the genotypes were evaluated for plant height, number of leaves per plant, relative water content (RWC), root length, number of lateral roots, SPAD value, pod length and pod yield were recorded at 60 days after sowing(DAS).

Genetic diversity analysis by ISSR molecular markers: To know the genetic diversity of the among the okra genotypes the genotypic variations was analysed by using DNA based ISSR molecular markers. Fresh Leaf samples were harvested and homogenized to fine powder in liquid nitrogen (-80°C) using mortar and pestle. Total DNA was extracted from fresh leaves of individual lines using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Annealing temperature for all the twenty ISSR primers was standardized by polymerase chain reaction.

PCR conditions: The test solutions were made up to a final volume of 20µl containing 50 ng of template DNA, 10 pM decamer primer, 1x reaction buffer, 0.1 mM dNTP mix and 1.0 U Taq DNA Polymerase. The amplification was performed using Eppendorf Thermocycler with a hot start for 2 minutes at 94ºC; followed by 35 cycles of denaturing at 94°C for 30 seconds; annealing for 30 seconds; and product extension for 5 minutes at 72°C. The PCR products after ISSR amplification were analyzed in 1.5% agarose gel containing Ethidium bromide electrophoresis system to resolve the different molecular configuration of a DNA molecule as well as to separate DNA fragments of different weights. DNA was stained by soaking the gel in a 0.5-mg/mL ethidium bromide solution and visualized under Gel documentation system. The genetic polymorphism was analyzed and the Dendrogram obtained from ntysis software for genetic similarity study of six okra lines.

RESULTS AND DISCUSSION

Laboratory Evaluation by PEG-6000

Germination (%): The seed germination percentage decreased with the decrease in water potential or by increasing PEG concentrations. The highest mean of germination percentage at all the PEG concentrations recorded in the genotypes Arka abhay (95.83) and P-8(95.83), whereas, least in Arka anamika (87.5%). However, interaction effect was non-significant (Table 1). Similar trend was observed by Larcher (2000).

SPAD values: The SPAD values was recorded at 22 days after sowing was decreased with increase of the water PEG-6000 stress levels. The mean SPAD value at PEG solutions with 0% and 15% PEG concentrations were 27.40 and 14.90 respectively. Significantly highest mean SPAD value at all the PEG concentrations recorded in Parbani kranti (24.17) followed by Arka abhay (22.79), whereas, least in Bagalkot local (19.08), however their interaction effect was non-significant .Decrease in chlorophyll content under drought

Table 1. Effect of PEG-6000 water stress on seed germination and SPAD values of okra

Genotype	Seed germination (%)					SPAD values at 22DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	96.67	96.67	96.67	93.33	95.83	27.73	24.47	20.60	15.17	21.99
White velvet	96.67	96.67	93.33	90.00	94.17	26.37	22.40	18.20	13.70	20.17
Arka Abhay	96.67	96.67	96.67	93.33	95.83	28.47	25.43	21.13	16.13	22.79
Parbhani Kranti	96.67	93.33	93.33	90.00	93.33	30.13	26.27	23.10	17.17	24.17
Arka Anamika	96.67	86.67	86.67	80.00	87.50	27.23	23.13	19.40	14.23	21.00
Bagalkot Local	96.67	96.67	93.33	86.67	93.33	24.47	21.60	17.27	13.00	19.08
Mean	96.67	94.44	93.33	88.89	93.33	27.40	23.88	19.95	14.90	21.53
For comparing				CD at 1%					CD at 1%	
Factor1				3.760					1.329	
Factor 2				NS					1.049	
Interaction				NS					NS	

stress conditions could be related to photo-oxidation resulting from oxidative stress which reduces the photosynthetic process in plants, results were in accordance with earlier findings (Ashraf 2009, Altaf et al 2015).

Shoot length (cm): The shoot length was decreased with increasing PEG concentration percentage. At 14 DAS the mean seed shoot length at PEG solutions with 0% and 15% PEG concentrations were 8.85cm and 7.46cm respectively. The significantly highest mean of shoot length at all the PEG concentrations in Parbani kranti (9.13) whereas, least in Bagalkot local (7.73). In their interaction effect the genotype Parbani kranti recorded significantly highest shoot length (9.60) among all the genotypes and in all the PEG concentrations. At 22DAS, significantly highest mean of shoot length at all the PEG concentrations was in the genotype Parbani kranti (11.73) whereas, least in Bagalkot local (9.50). The genotype Parbani kranti recorded significantly highest shoot length (13.40) in all the PEG concentrations (Table 2). Bhatt and Rao, (2005 observed that reduction in shoot length and plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress.

Root length (cm): The root length was increased with the decrease in water potential (or by increasing PEG concentration). At 14DAS, significantly highest mean of root length at all the PEG concentrations recorded in the genotype Parbani kranti (12.82) whereas, least in Bagalkot local (11.45). In their interaction effect the genotype Parbani kranti recorded significantly highest root length (14.70). At 22DAS, there was significantly highest root length was recorded in Arka abhay (22.85) whereas, least in Arka anamika (19.63). In their interaction, Arka abhay recorded significantly highest root length (25.10) (Table 3). Such increase in linear growth of roots is possessed by either increase of gibberlins or cytokinins or to the ability of roots to branch and elongate quickly in try to acquire underground water to tolerate the stress conditions, which thus enable plants to survive properly irrespective of water stress. Similar results were also reported by Kader et al (2014) and Hasan et al (2018).

Number of lateral roots: The number of lateral roots was increased with the increasing PEG concentration. At 14DAS, significantly highest mean of number of lateral roots at all the PEG concentrations recorded in Parbani kranti (12.82) whereas, least in Bagalkot local (11.45). In their interaction effect also Parbani kranti recorded significantly highest number of lateral roots (27.90) among all the genotypes and in all the PEG concentrations. At 22DAS, significantly highest mean of number of lateral roots at all the PEG concentrations recorded in Arka abhay (33.48) whereas, least in white velvet local (29.88). In their interaction effect also Arka abhay (37.00) was significantly more number of lateral roots (Table 4).

Relative water content: The leaf relative water content (RWC %) was decreased with the increasing PEG concentrations. At 14DAS, significantly highest RWC % recorded in Arka abhay (81.58) whereas, least in Bagalkot local (76.05). In their interaction effect Arka abhay recorded significantly highest RWC % (90.50) among all the genotypes and in all the PEG concentrations compared to others. At 22DAS, significantly highest mean RWC % at all the PEG concentrations recorded in P-8 (73.30) followed by Parbani kranti (72.45), whereas, least in Bagalkot local (71.03). In their interaction effect P-8(87.6) recorded significantly

Table 2. Effect of PEG-6000 water stress on shoot length of okra at 14 and 22 days after sowing

Genotype	Shoot length at 14 DAS					Shoot length at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	9.20	9.00	9.00	8.00	8.80	12.20	11.80	10.90	9.50	11.10
White velvet	8.90	8.70	8.60	7.80	8.50	11.77	11.60	10.50	9.20	10.77
Arka Abhay	8.50	8.20	8.00	6.90	9.13	11.00	10.83	9.90	8.60	10.08
Parbhani Kranti	9.60	9.40	9.20	8.33	9.13	13.40	12.10	11.30	10.10	11.73
Arka Anamika	8.70	8.40	8.17	7.40	8.17	11.27	11.07	10.20	8.87	10.35
Bagalkot Local	8.20	8.20	8.20	6.30	7.73	10.20	10.50	9.60	7.70	9.50
Mean	8.85	8.65	8.53	7.46	8.58	11.64	11.32	10.40	8.99	10.6
For comparing				CD at 1%					CD at 1%	
Factor1				0.207					0.123	
Factor 2				0.159					0.289	
Interaction				0.326					0.536	

highest RWC % compared to others genotypes (Table 5). This decline of relative water content is consistent with earlier studies (Zhang et al 2010, Prabhakar et al 2018).

Field evaluation by induced water stress: Six okra genotypes were evaluated for drought tolerance under water stress condition (65% field capacity) and well irrigated condition as control.

Plant height (cm): At 60 DAS there was a significance difference for genotypes and water levels and their interaction effect. There was significantly higher mean plant height in well irrigated condition (63.0) compared to water stress condition (54.7). In well irrigated regime among the genotypes Arka anamika (64.4) showed significantly higher plant height whereas, least recorded in Bagalkot local (59.6). In water stress regime among the genotypes White velvet (57.8) showed significantly higher plant height whereas, least in Bagalkot local (51.8). In their interaction effect, Arka anamika (64.4) recorded significantly highest plant height among all genotypes and in both the water regime levels (Table 6). Altaf et al (2015) observed that drought reduced the plant height and maximum reduction in plant height was observed in highest levels of drought (50%) than the lower levels and concluded that drought up to 50% could be fatal for okra but plant can survive at low level of drought. Reduction in plant height under severe moisture stress, could be due to decrease in cell elongation and cell division so it gradually reduces leaf area. The results are in agreement with the results obtained by Kader et al (2010).

Table 3. Effect of PEG-6000 water stress on root length(cm) in okra at 14 and 22 days after sowing

Genotype	Root length(cm) at 14 DAS					Root length(cm) at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	11.00	11.17	13.20	12.37	11.93	19.00	20.40	22.00	24.00	21.35
White velvet	10.30	10.20	12.27	13.80	11.64	18.20	19.90	21.20	22.70	20.50
Arka Abhay	11.90	12.80	12.00	13.40	12.53	21.60	21.30	23.40	25.10	22.85
Parbhani Kranti	11.40	11.50	13.67	14.70	12.82	19.60	20.70	22.50	24.20	21.75
Arka Anamika	9.70	10.00	12.80	14.20	11.68	17.00	18.60	20.60	22.30	19.63
Bagalkot Local	10.60	10.70	11.50	13.00	11.45	18.70	20.20	21.60	23.23	20.93
Mean	10.82	11.06	12.57	13.58	12.0	19.02	20.18	21.88	23.59	21.2
For comparing				CD at 1%					CD at 1%	
Factor1				0.297					0.675	
Factor 2				0.221					0.416	
Interaction				0.458					0.905	

Table 4. Effect of PEG-6000 water stress on number of lateral roots at 14 and 22 days after sowing

Genotype	Number of lateral roots at 14 DAS					Number of lateral roots at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	20.60	23.00	24.50	26.70	11.93	27.80	30.50	32.00	35.57	31.47
White velvet	18.93	22.23	23.60	25.70	11.64	26.20	28.73	31.00	33.60	29.88
Arka Abhay	22.40	24.83	25.40	27.90	12.53	30.60	32.30	34.00	37.00	33.48
Parbhani Kranti	20.00	23.40	24.80	27.23	12.82	28.63	31.00	32.80	36.10	32.13
Arka Anamika	18.33	21.67	23.10	25.00	11.68	25.10	28.00	29.70	32.00	28.70
Bagalkot Local	19.33	22.60	23.97	26.10	11.45	27.00	29.77	31.53	34.73	30.76
Mean	19.93	22.96	24.23	26.44	12.0	27.56	30.05	31.84	34.83	31.07
For comparing				CD at 1%					CD at 1%	
Factor1				0.629					0.237	
Factor 2				0.384					0.364	
Interaction				0.769					0.687	

Number of Leaves per plant: There was significantly higher mean number of leaves per plant at in well irrigated condition (13.5) compared to water stress condition (12.4). In well irrigated regime among the genotypes White velvet (14.4) showed significantly higher number of leaves per plant whereas, least recorded in Bagalkot local (12.4). In water stress regime also among the genotypes White velvet (13.1) showed significantly higher number of leaves per plant whereas, least recorded in Bagalkot local (11.4). In their interaction effect the genotype White velvet (14.4) recorded significantly highest number of leaves per plant among all genotypes and in both the water regime levels (Table 6.).

Leaf Relative water content (RWC%): There was significantly higher mean RWC % in well irrigated condition (78.4) compared to water stress condition (71.1). In well irrigated regime among the genotypes Arka abhay (85.0) showed significantly higher RWC % whereas, least was in Aarka anamika (72.3). In water stress regime also among the genotypes, Arka abhay (75.3) showed significantly higher RWC % whereas, least recorded in Arka anamika (67.1). In their interaction effect the genotype Arka abhay (85.0) recorded significantly highest RWC % among all genotypes and in both the water regime levels compared to others (Table 7). The reduction of relative water content under moderate and severe stress is probably an oxidative injury at the cellular level under water stress has high lipid peroxidation which decrease the stability of cell membrane and led to lose more water from cells.

Root length (cm): There was significantly higher mean root length in water stressed regime condition (30.7) compared to

Table 5. Effect of PEG-6000 water stress on Leaf RWC (%) in okra at 14 and 22 days after sowing

Genotype	Relative water content (%) at 14 DAS					Relative water content (%) at 22 DAS				
	PEG 6000 concentration (%)				Mean	PEG 6000 concentration (%)				Mean
	0	5	10	15		0	5	10	15	
P-8	88.17	82.00	76.40	71.90	79.62	87.60	76.60	67.40	61.60	73.30
White velvet	87.40	80.80	74.80	66.90	77.48	84.70	74.70	66.40	60.60	71.60
Arka Abhay	90.50	84.20	78.10	73.50	81.58	85.33	75.60	66.80	60.90	72.16
Parbhani Kranti	89.40	82.87	77.20	72.60	80.52	85.70	75.90	67.00	61.20	72.45
Arka Anamika	85.00	80.40	73.10	65.70	76.05	85.10	75.10	66.60	60.70	71.88
Bagalkot Local	86.60	81.47	75.50	68.20	77.94	84.40	73.60	65.80	60.30	71.03
Mean	87.84	81.96	75.85	69.80	78.9	85.47	75.25	66.67	60.88	72.1
For comparing				CD at 1%					CD at 1%	
Factor1				0.249					0.295	
Factor 2				0.391					0.449	
Interaction				0.736					0.847	

Table 6. Effect of water stress on plant height and number of leaves per plant in okra

Genotype	Plant height (cm) at 60 DAS			Number of leaves per plant at 60 DAS		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	63.8	55.3	59.5	13.4	12.6	13.0
White velvet	66.0	57.8	61.9	14.4	13.1	13.8
Arka Abha	62.8	53.8	58.3	14.3	12.2	13.2
Parbhani Kranti	61.5	52.6	57.1	13.0	12.2	12.6
Arka Anamika	64.4	56.9	60.7	13.8	12.8	13.3
Bagalkot Local	59.6	51.8	55.7	12.4	11.4	11.9
Mean	63.0	54.7	58.9	13.5	12.4	13.0
For comparing			CD at 5%			CD at 5%
Main			0.688			0.352
Sub			0.388			0.205
M × S			0.602			0.405

well irrigated condition (27.9). In well irrigated regime among the genotypes Arka abhay (31.8) showed significantly higher root length whereas, least recorded in Aarka anamika (24.6). In water stress regime, Arka abhay (33.2) showed significantly higher root length compared to others whereas, least recorded in Arka anamika (28.1). In their interaction effect also Arka abhay (31.8) recorded significantly higher root length (Table 7). Hasan et al (2018) observed, increase in root length under drought treatment. Generally, the root length of plants increases during a water stress conditions because the plants try to acquire underground water to tolerate the stress condition, in line with this, the root length is greater in drought tolerant species compared to sensitive species.

Number of lateral roots:. There was significantly higher mean number of lateral roots in water stressed condition (30.2) compared to well irrigated condition (27.5). In well irrigated regime among the genotypes Arka abhay (30.4) showed significantly higher number of lateral roots whereas, least recorded in Aarka anamika (25.1). In water stress regime, Arka abhay (34.3) showed significantly higher number of lateral roots whereas, least recorded in Arka anamika (27.0). In their interaction effect also Arka abhay (34.3) recorded significantly more number of lateral roots (Table 7). Reduction in roots, root diameter under severe moisture stress, could be due to decrease in cell elongation. The results of our study are in accordance with the findings of Hasan et al (2018).

SPAD Value: There was significantly highest mean SPAD value in well irrigated condition (26.1) compared to water stressed condition (22.1). In well irrigated regime among the genotypes Arka abhay (30.2) showed significantly higher SPAD value whereas, least recorded in Aarka anamika (23.0). In water stress regime, Arka abhay (25.8) showed significantly higher SPAD value whereas, least recorded in Arka anamika (17.2). In their interaction effect also significant (Table 7).

Pod length (cm): There was significantly highest mean pod length in well irrigated condition (10.7) compared to water stressed condition (9.9). In well irrigated regime among the genotypes Parbani kranthi (11.4) showed significantly higher pod length whereas, least recorded in Bagalkot local (9.6). In water stress regime, P-8 (11.5) showed significantly higher pod length whereas, least recorded in Bagalkot local (8.4). In their interaction effect also P-8 (11.5) significantly recorder higher pod length (Table 8). When okra plant exposed to 50 % level of drought reduces the growth and photosynthetic pigment which resulting in reduction of pod yield gradually. In the same line, length and number of pods per plant showed positive direct effect on pod yield.

Pod Yield (t/ha): There was significantly highest mean pod yield in well irrigated condition (18.19) compared to water stressed condition (14.81). In well irrigated regime among the genotypes P-8 (19.46) showed significantly higher pod yield whereas, least recorded in Bagalkot local (16.72). In water stress regime also, P-8 (15.54) showed significantly higher pod yield whereas, least recorded in Bagalkot local (14.02). In their interaction effect also P-8 (19.46) significantly recorded higher pod yield (Table 8).

Genetic diversity analysis by ISSR markers: To genetic diversity was analysed for six okra genotypes by using twenty ISSR genetic markers (Table 9). Initially DNA from leaf was

Table 7. Effect of water stress on Leaf Relative water content(%), Root length(cm) and Number of lateral roots in okra at 60 DAS

Genotype	Leaf Relative water content (%)			Root length (cm)			Number of lateral roots		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	79.4	73.0	76.2	28.2	31.2	29.7	27.9	30.4	29.2
White velvet	74.5	68.8	71.7	26.4	29.2	27.8	26.2	28.7	27.5
Arka Abhay	85.0	75.3	80.2	31.8	33.2	32.5	30.4	34.3	32.4
Parbhani Kranti	82.5	72.0	77.3	29.2	32.1	30.7	28.7	31.8	30.3
Arka Anamika	72.3	67.1	69.7	24.6	28.1	26.4	25.1	27.0	26.1
Bagalkot Local	76.4	70.3	73.4	27.1	30.2	28.6	26.8	29.2	28.0
Mean	78.4	71.1	74.7	27.9	30.7	29.3	27.5	30.2	28.9
For comparing			CD at 5%			CD at 5%			CD at 5%
Main			1.327			0.717			0.225
Sub			1.034			0.585			0.235
M × S			1.48			0.831			0.325

Table 8. Effect of water stress on SPAD value, pod length and pod yield in okra at 60 DAS

Genotype	SPAD value			Pod length (cm)			Pod yield (t/ha)		
	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean	Well irrigated	Water stressed	Mean
P-8	26.2	23.4	24.8	11.9	11.5	11.7	19.46	15.54	17.75
White velvet	25.4	20.1	22.7	11.0	10.1	10.6	18.46	14.93	17.65
Arka Abhay	30.2	25.8	28.0	10.4	9.8	10.1	18.19	14.70	17.61
Parbhani Kranti	27.0	24.5	25.8	11.4	10.6	11.0	18.73	15.22	17.62
Arka Anamika	23.0	17.2	20.1	10.0	9.2	9.6	17.58	14.44	17.53
Bagalkot Local	24.5	21.7	23.1	9.6	8.4	9.0	16.72	14.02	17.36
Mean	26.1	22.1	24.1	10.7	9.9	10.3	18.19	14.81	17.59
For comparing			CD at 5%			CD at 5%			CD at 5%
Main			1.14			0.225			0.036
Sub			0.928			0.235			0.049
M × S			1.319			0.325			0.065

Table 9. List of ISSR primers used for genetic diversity study with their sequence

Primer name	Sequence (5'-3')	Sl. No	Primer name	Sequence (5'-3')
UBC811	GAG AGA GAG AGA GAG AC	11	SPS7	$(GTG)_5$
UBC841	GAG AGA GAG AGA GAG AYC	12	SPS1	$(GAC)_5$
UBC826	ACA CAC ACA CAC ACA CC	13	I3	$(GA)_9A$
UBC818	CAC ACA CAC ACA CAC AG	14	ISSR1	$(CT)_8$ TG
UBC834	AGA GAG AGA GAG AGA GYT	15	ISSR2	$(CT)_8$ AC
UBC835	AGA GAG AGA GAG AGA GYC	16	ISSR4	$(AGC)_4$ GT
UBC850	GTG TGT GTG TGT GTG TYC	17	ISSR5	$(CAC)_3GC$
I2	$(GA)_9T$	18	ISSR6	$(CTC)_3$ GC
SPS8	$(GGA)_4$	19	ISSR7	$(GACA)_3$
SPS4	$(AGG)_6$	20	ISSR8	$(GACA)_3$ GC

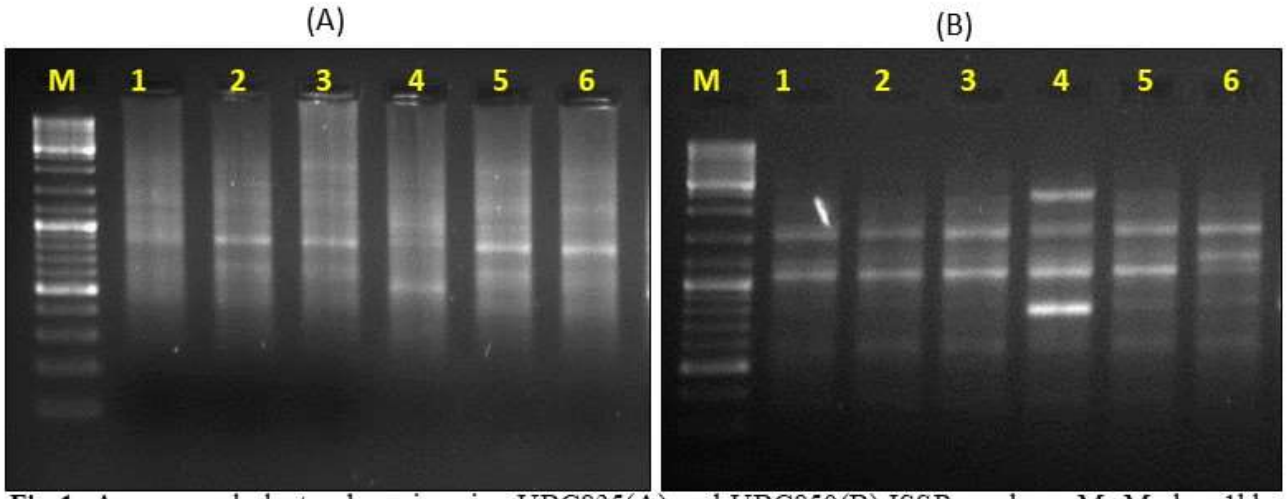

Fig 1. Agarose gel electrophoresis using UBC835(A) and UBC850(B) ISSR markers. M- Marker 1kb ladder, Lane 1-6 Okra lines (1- P-8, 2-White velvet, 3-Arka abhay, 4-Parbani kranti, 5-Arka anamika and 6- Bagalkot local)

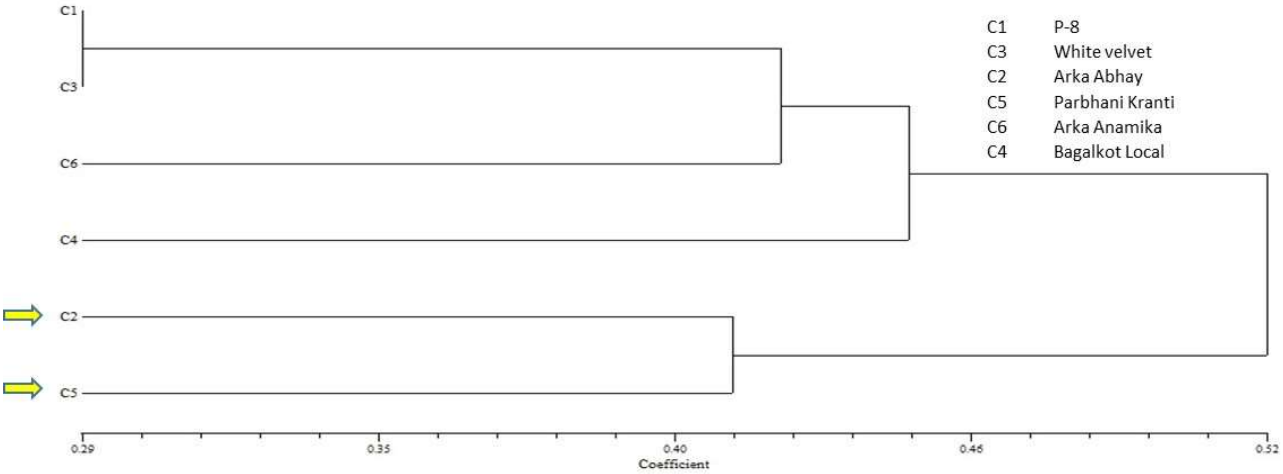

Fig 2. Dendrogram depicting the six Okra lines based on the genetic similarity generated by ISSR markers

isolated from using CTAB method and quantified using Nanadrop. Annealing temperature for all the ISSR primers was standardized by PCR (polymerase chain reaction). PCR performed for all the 20 primers to amplify to arrive allelic variations. Out of twenty primers eleven primers showed polymorphisms (Fig 1). The results showed that moderate genetic polymorphism (29.0%) among okra genotypes. Dendrogram obtained from ntysis software grouped the six okra lines into different clusters. The relatively water stress tolerant lines (Arka abhay and Parbani kranti) were clustered in one group and the rest were grouped in other cluster (Fig. 2).

CONCLUSION

Based on laboratory evaluation PEG-6000 induced water stress and field stress it is revealed that among six okra genotypes Arka abhay followed by Parbani kranti were relatively drought tolerant by exhibiting physiological drought adoptive traits like high relative water content, RWC, SPAD, root traits with better plant height.

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