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EVALUATION AND IDENTIFICATION OF TOBACCO BREEDING LINES FOR WATER STRESS TOLERANCE

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Tobacco (*Nicotiana tabacum* L.) is an important industrial crop grown in the states of Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka, West Bengal, Bihar and Uttar Pradesh. The tobacco crop is relatively drought stress tolerant plant but the loss of quality leaf yield in response to water-deficit stress is witnessed in many areas. For selection of drought tolerant genotype the lines need to be screened under artificial condition to ensure that all the genotypes undergo drought stress. In this study artificial water stress has been created for screening the genotypes under *in vitro* and *in vivo* condition and the genotypes were selected based on morphological and physiological characteristics. The minimum germination per cent with 1% PEG was found to be 10% and maximum was 90%. Twenty five genotypes showed 90 % germination under stress whereas the susceptible check showed only 10 % germination. In greenhouse, water stress was induced at 35 days after transplantation in pots and the soil moisture (SM) and relative water content (RWC) were recorded three times at weekly intervals. RWC of tolerant lines varied from 40-50 per cent when the soil moisture was below 20%. The height of the genotypes varied from 80 to 100 cm, whereas the height of the susceptible check was 34 cm. A total of 12 genotypes were selected based on the RWC which showed a balanced RWC during three intermittent water stress and the recorded higher photosynthetic rate between 12-18 mmol CO_2 $m^{-2}s^{-1}$, stomatal conductance 0.2-0.8 H_2O m^{-2} s^{-1}, transpiration rate 6- 9 H_2O m^{-2} s^{-1} and Ci/Ca ratio 0.6- 0.797.

Key words: Drought, Germination Percentage, PEG, Relative water content, tobacco

Introduction

Abiotic stress hampers the realization of full genetic potential of crops and has major impact on agricultural crop quality and productivity. Among various abiotic stresses, drought causes heavy damage to crops. The erratic rainfall and unpredictable climate changes always remains as a challenge to the farming community. During prolonged drought conditions the reduction in water content in the plant system results in reduced leaf water potential, turgor pressure thereby leading to stomatal closure which in turn affects the overall growth and development of the plant. As the drought period extends the photosynthesis and metabolism of the plant system is disturbed finally leading to death of the plants (Jaleel *et al.*, 2008a). Understanding the morphological and physiological changes in the plant system when the plant is under drought stress helps the researcher to screen the genotypes that can with stand water deficit.

Tobacco (*Nicotiana tabacum* L.) belonging to Solanaceae family is an important industrial crop and is one of the most widely cultivated non-food crops worldwide and is grown in approximately 120 countries. In India it is grown in the states of Andhra Pradesh, Gujarat, Tamil Nadu, Karnataka, West Bengal, Bihar and Uttrapradesh. In most of the areas tobacco is cultivated with conserved soil moisture during *rabi* or as rainfed crop in *Kharif*. Hence, rainfall directly affect the crop growth and yield. When rainfall is low and erratic, the crop is subjected to water stress. Even though the tobacco is relatively drought stress tolerant plant per se, studies demonstrated the loss of quality leaf yield in response to water-deficit stress (Biglouei *et al* 2010; Cakir *et al.*, 2010; Riga & Vartanian, 1999; Tsai & Maw 1988; Celik & Atak. 2012). Development of drought tolerant tobacco varieties can withstand the drought without loss of leaf yield.

In view of the frequent drought spells observed in southern light soils of tobacco growing regions of Andhra Pradesh and its impact on tobacco yields, breeding programme initiated for developing water stress tolerant tobacco genotypes. Some of the selected breeding lines developed were screened under artificial water stress conditions. The present paper deals with the response of these genotypes to artificial water stress created under *in vitro* and *in vivo* condition for various morphological and physiological characteristics.

MATERIALS AND METHODS

This present study was conducted at ICAR-Central Tobacco Research Institute (CTRI), Rajahmundry during 2017 *rabi*. Forty seven breeding lines (F_4 generation), developed from the cross combination of MRS 3 X Siri along with the parents were used in the study. MRS 3, drought tolerant genotype, maintained at ICAR-CTRI, was used as donor parent. Owing to its drought tolerance nature and good performance under stress conditions, it could serve as an important source for mining drought tolerant QTLs. Siri used as recipient parent is a high yielding variety but very much prone to drought. Selected F_4 generation lines were screened in *in vitro* and *in vivo* conditions along with the parents to study the performance of genotypes under drought conditions.

In vitro: The seeds of F_4 generation along with the parents were germinated in petriplates in two replications, one with normal water and other with 1 per cent poly ethylene glycol (PEG) (molecular weight- 6000-7500) and the germination percentage was recorded.

In vivo: The crop was raised in greenhouse during *kharif* 2017 and was subjected to drought stress (DS) along with irrigated non-stress (NS) control. Four replications were maintained. The seeds were grown in plastic pots of 10 x 15 cm filled with red soil and sand. The seedlings were transplanted in pots of 25 cm height x 20 cm diameter with drainage hole filled with three parts of coir pith and one part of natural clay loam soil. Three plants per pot were maintained and were grown in green house under normal temperature. The crop was irrigated till 35^{th} day after transplanting and there after irrigation was stopped for two replications at peak vegetative stage. Soil moisture (SM) was recorded at regular intervals and after the SM reached below 20% in the stress treatments, the crop was irrigated. Thus three cycles of intermittent severe stress was imposed until the reproductive stage. Various physiological and morphological observations were recorded to study the response of the genotype under drought for selecting the better performing lines for further studies.

Statistical analysis: All the morphological and physiological data were analysed using XLStat-2018.

RESULTS AND DISCUSSION

The parent and F_4 generation seeds were germinated in 1% PEG. Comparative studies between control and stress condition showed that there was difference in the germination per cent of the seeds under both the conditions. The minimum germination per cent under 1% PEG was found to be 10% and maximum was 90 % (Table. 1). The stress tolerant parent, MRS-3 recorded 90% germination and Siri, susceptible one recorded 10%. Among the breeding lines, 26 genotypes showed 90 % germination under stress as that of tolerant parent (ure. 1) indicating that these lines are tolerant to water stress.

Table 1: Germination percentage of tobacco genotypes under control and 1% PEG treatments

Genotypes	Germination (%)	
	Control	1% PEG
MRS-3	90	90
Siri	90	10
MRS 3x SIRI # 1	90	90
MRS 3x SIRI # 2	90	90
MRS 3x SIRI # 3	90	90
MRS 3x SIRI # 4	90	90
MRS 3x SIRI # 5	90	90
MRS 3x SIRI # 6	90	90
MRS 3x SIRI # 7	90	90
MRS 3x SIRI # 8	90	90
MRS 3x SIRI # 9	90	90
MRS 3x SIRI # 10	90	90
MRS 3x SIRI # 11	90	90
MRS 3x SIRI # 12	90	90
MRS 3x SIRI # 13	90	90

MRS 3x SIRI # 14	90	90
MRS 3x SIRI # 15	90	90
MRS 3x SIRI # 16	90	90
MRS 3x SIRI # 17	90	90
MRS 3x SIRI # 18	90	90
MRS 3x SIRI # 19	90	90
MRS 3x SIRI # 20	90	90
MRS 3x SIRI # 21	90	90
MRS 3x SIRI # 22	90	50
MRS 3x SIRI # 23	90	50
MRS 3x SIRI # 24	90	50
MRS 3x SIRI # 25	90	50
MRS 3x SIRI # 26	90	90
MRS 3x SIRI # 27	80	50
MRS 3x SIRI # 28	80	50
MRS 3x SIRI # 29	90	90
MRS 3x SIRI # 30	70	30
MRS 3x SIRI # 31	80	80
MRS 3x SIRI # 32	90	50
MRS 3x SIRI # 33	80	80
MRS 3x SIRI # 34	50	20
MRS 3x SIRI # 35	50	50
MRS 3x SIRI # 36	90	50
MRS 3x SIRI # 37	80	80
MRS 3x SIRI # 38	80	80
MRS 3x SIRI # 39	90	90
MRS 3x SIRI # 40	60	90
MRS 3x SIRI # 41	90	30
MRS 3x SIRI # 42	80	10
MRS 3x SIRI # 43	90	10
MRS 3x SIRI # 44	80	20
MRS 3x SIRI # 45	95	90
MRS 3x SIRI # 46	90	50
MRS 3x SIRI # 47	90	40
Mean	85.82	69.80
Variance (n-1)	96.32	675.67
SD(n-1)	9.55	26.76
Max	95.00	90.00
Min	50.00	10.00

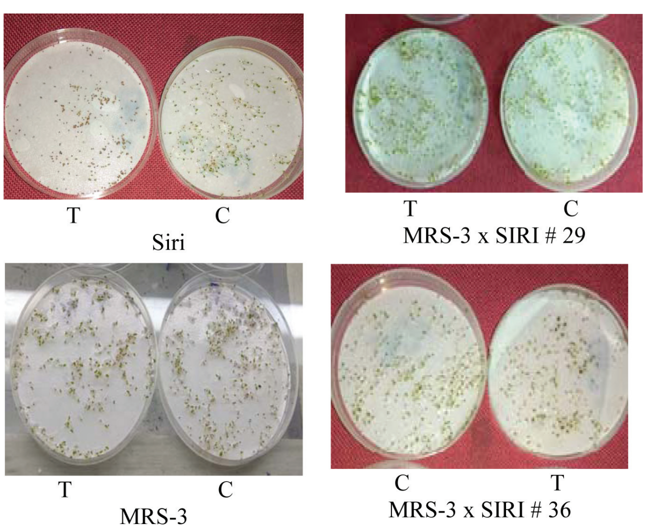

Fig. 1: Seed germination of Siri, MRS-3 and breeding lines under control and water stress (with 1% PEG solution)

The breeding lines and parents were raised in pots in greenhouse and water stress was induced at 35 days after transplantation. After first irrigation succeeding the stress the susceptible lines recovery was very slow whereas the tolerant plants could overcome the shock and recover rapidly. After three cycles of intermittent drought the yield related observations were recorded only in the tolerant plants along with the parents. SM and RWC were recorded three times at an interval of a week gap. Though Siri recorded higher RWC at the start of stress, MRS-3 recorded higher RWC than Siri once the stress progressed indicating the ability of MRS-3 to adopt to stress (Table 2). MRS 3x SIRI # 32 recorded higher RWC (92.46%) and MRS 3x SIRI # 26 lowest (80.80%) at the initiation of the stress. In general RWC found to decrease gradually in all the genotypes after the stress treatment as evidenced from the observations recoded at time intervals. RWC of lines varied from 30.30-51.60 per cent when the soil moisture was below 20% at around 15 days of stress treatment. The plants maintaining higher RWC under moisture stress has the ability to overcome drought stress (Baghyalakshmi *et al.*, 2016) and hence 12 genotypes recording significantly higher RWC than mean are selected and for further study with respect to various morphological and physiological character. Seed germination of all these selected genotypes under stress (with PEG 1%) found to be comparable to control.

Table 2: Relative water content (RWC) in tobacco genotypes at different time intervals during stress

Genotypes	RWC after water stress initiation		
	0^{th} DAS	7^{th} DAS	15^{th} DAS
MRS-3	88.92	75.46	48.8
Siri	90.23	75.11	38.2
MRS 3x SIRI # 1	89.09	74.77	35.8
MRS 3x SIRI # 2	88.76	72.34	**47.9**

MRS 3x SIRI # 3	89.89	81.81	30.6
MRS 3x SIRI # 4	89.83	82.23	38.9
MRS 3x SIRI # 5	90.31	78.46	**51.6**
MRS 3x SIRI # 6	89.77	81.39	**50.7**
MRS 3x SIRI # 7	90.40	80.87	37.4
MRS 3x SIRI # 8	90.02	82.00	35.8
MRS 3x SIRI # 9	88.01	81.43	38.2
MRS 3x SIRI # 10	89.65	76.94	38.4
MRS 3x SIRI # 11	89.67	78.71	**47.4**
MRS 3x SIRI # 12	89.12	78.40	**45.9**
MRS 3x SIRI # 13	90.57	77.30	38.3
MRS 3x SIRI # 14	88.56	78.55	38.9
MRS 3x SIRI # 15	86.53	79.97	37.3
MRS 3x SIRI # 16	89.24	80.07	**45.3**
MRS 3x SIRI # 17	90.19	79.22	38.3
MRS 3x SIRI # 18	90.72	80.18	31.8
MRS 3x SIRI # 19	89.57	80.98	30.7
MRS 3x SIRI # 20	89.82	77.25	38.4
MRS 3x SIRI # 21	90.29	81.45	**45.3**
MRS 3x SIRI # 22	89.35	80.52	43.0
MRS 3x SIRI # 23	81.28	70.40	38.6
MRS 3x SIRI # 24	81.41	75.37	36.9
MRS 3x SIRI # 25	81.35	70.96	35.8
MRS 3x SIRI # 26	80.80	71.51	**48.8**
MRS 3x SIRI # 27	87.54	78.69	38.3
MRS 3x SIRI # 28	86.03	78.90	37.3
MRS 3x SIRI # 29	85.82	79.64	**48.6**
MRS 3x SIRI # 30	85.60	78.80	36.9
MRS 3x SIRI # 31	87.91	76.31	38.3
MRS 3x SIRI # 32	92.46	82.06	37.4
MRS 3x SIRI # 33	86.61	76.95	**45.9**
MRS 3x SIRI # 34	87.26	77.59	37.4
MRS 3x SIRI # 35	88.44	76.63	30.5
MRS 3x SIRI # 36	88.93	74.43	29.2
MRS 3x SIRI # 37	89.41	74.16	37.3
MRS 3x SIRI # 38	85.06	74.71	35.7
MRS 3x SIRI # 39	87.40	75.94	**48.7**
MRS 3x SIRI # 40	86.67	75.71	38.7
MRS 3x SIRI # 41	89.96	75.47	30.3
MRS 3x SIRI # 42	87.04	73.80	30.1
MRS 3x SIRI # 43	89.07	78.78	35.0
MRS 3x SIRI # 44	84.84	79.32	38.7
MRS 3x SIRI # 45	88.54	74.43	**48.9**
MRS 3x SIRI # 46	89.59	78.23	45.4
MRS 3x SIRI # 47	87.55	76.32	37.1
Mean	88.06	77.56	39.56
Variance (n-1)	7.04	9.46	36.03
SD(n-1)	2.59	3.02	5.97
Max	92.46	82.23	51.61
Min	80.80	70.40	29.15

* Figures in bold indicates the selected genotypes for further studies

Morphological observations were recorded after the completion of three cycles of water stress under both control and stress conditions in the selected genotypes and parents (Table 3). Siri recorded lowest RWC values after each cycle of drought treatment compared to MRS-3 and breeding lines. The RWC values after the second cycle are relatively higher than the other values, due to the cloudy weather prevailed during the period. In general, the plant height, number of leaves, length and width of leaves in bothe parents and breeding lines reduced in stress condition compared to stress. The height of Siri, susceptible parent was about 35 cm under water stress

Fig. 2: Parents and breeding lines under control and drought condition

condition and in the breeding lines it varied from 80 to 100 cm. All the breeding lines showed more height when compared to Siri under stress and on par with the tolerant parent. The reduction in height in stress condition when compared to controlled condition was found to be in a range of 25-36% in the breeding lines whereas the susceptible parent showed about 75% reduction in plant height. The tolerant genotypes could yield a minimum of 12 to 16 broad leaves under stress condition when compared to Siri which had only 5 curable leaves under stress condition but 26 leaves in controlled condition (Table 3). The reduction percent in the number of leaves in the susceptible parent (81%) was much higher when compared to MRS-3 (45%). Similarly, the width and length of the leaves in susceptible parent was higher (62 and 36% reduction, respectively) when compared to that of tolerant parent (25% and 14%) and the selected genotypes (23-44% and -4.3-17%, respectively).

Further, physiological observations like photosynthetic rate, conductance, transpiration and ci/ca ratio were recorded to confirm the tolerance of selected genotypes under drought situations (Table 4). All the 12 genotypes showed a balanced RWC during three intermittent water stresses and the recorded higher photosynthetic rate between (12-18 mmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (0.2-0.8 H_2O m^{-2} s^{-1}), transpiration rate (6- 9 H_2O m^{-2} s^{-1}) and Ci/Ca ratio (0.6- 0.797) compared to susceptible parent, Siri (4.321 CO_2 $m^{-2}s^{-}$, 0.011 H_2O m^{-2} s^{-1}, 3.312^1H_2O m^{-2} s^{-1}, 0.029) (Figure 3, 4) showing the plasticity of breeding lines to withstand drought situations. The results obtained by Tezera *et al.* (2002) reported that higher stomatal conductance would result in higher photosynthetic rate and biomass production. As it is a well known fact that higher biomass is a requisite for better yields in tobacco crop, hence the plants with good photosynthetic rate under drought has to be selected. Martinez *et al.* (2007) has reported in his study that higher stomatal conductance may be an enhanced adaptation of plants to drought environments. Chen *et al.* (1995) observed that elevating photosynthetic rate is beneficial to dry matter production and yield.

Table 3: Morphological characters of selected tobacco genotypes under water stress

Genotypes	Plant height			Number of leaves			Length of leaves			Width of leaves		
	Control	Stress	R%	Control	Stress	R%	Control	Stress	R%	Control	Stress	R%
MRS 3	123	87	29.27	22	12	45.45	49.6	37	25.40	21.2	18.2	14.15
SIRI	139	34.7	75.04	26	5	80.77	57.4	22	61.67	25.4	16.2	36.22
MRS 3x SIRI # 2	132	92	30.30	24	14	41.67	52.3	36	31.17	23.6	22.4	5.08
MRS 3x SIRI # 5	129	84.2	34.73	23	11	52.17	56.1	32.5	42.07	22.9	20.3	11.35
MRS 3x SIRI # 6	136	96.3	29.19	25	16	36.00	55.7	31.4	43.63	23.5	20.1	14.47
MRS 3x SIRI # 11	133	94.5	28.95	24	15	37.50	56.3	36.2	35.70	24	22.7	5.42
MRS 3x SIRI # 12	128	82	35.94	22	9	59.09	51.8	32.2	37.84	23.8	19.8	16.81
MRS 3x SIRI # 16	135	95.1	29.56	25	14	44.00	53.4	34.3	35.77	25.3	22.1	12.65
MRS 3x SIRI # 21	129	94.9	26.43	22	14	36.36	49.8	38	23.69	23.2	24.2	-4.31
MRS 3x SIRI # 26	124	92.5	25.40	20	13	35.00	51.2	39.2	23.44	25.1	24.3	3.19
MRS 3x SIRI # 29	131	95	27.48	25	15	40.00	57.1	36.1	36.78	24.2	23.6	2.48
MRS 3x SIRI # 33	126	86	31.75	22	12	45.45	54.5	37.4	31.38	23.9	23.8	0.42
MRS 3x SIRI # 39	132	94.7	28.26	24	16	33.33	55.2	34.3	37.86	24.6	21.9	10.98
MRS 3x SIRI # 45	127	85.9	32.36	23	10	56.52	51.7	36.2	29.98	23.7	23.5	0.84
Minimum	123	34.7		22	5		49.6	22		21.2	16.2	
Maximum	139	96.3		26	16		57.4	39.2		25.4	24.3	
Mean	130.3	86.8		23.4	12.6		53.7	34.5		23.9	21.7	
Variance (n-1)	13.24	247.61		2.39	9.34		5.64	18.06		0.52	5.89	
SD(n-1)	3.64	15.74		1.54	3.06		2.38	4.25		0.72	2.43	

Table 4. Physiological characters of selected genotypes

Genotypes	RWC			3rd cycle			
	1st cycle	2nd cycle	3rd cycle	PR	SC	TR	Ci/Ca
MRS 3	48.8	52.68	45.26	12.012	0.254	7.261	0.573
SIRI	38.2	35.32	33.24	4.321	0.011	3.312	0.029
MRS 3x SIRI # 2	47.9	57.72	46.44	13.260	0.255	7.468	0.559
MRS 3x SIRI # 5	51.6	57.28	48.36	12.856	0.474	6.083	0.627
MRS 3x SIRI # 6	50.7	58.30	46.46	18.887	0.350	9.325	0.589
MRS 3x SIRI # 11	47.4	56.93	46.40	15.761	0.293	8.098	0.555
MRS 3x SIRI # 12	45.9	57.89	46.37	11.138	0.259	7.117	0.598
MRS 3x SIRI # 16	45.3	56.79	47.25	17.928	0.201	8.826	0.496
MRS 3x SIRI # 21	45.3	58.78	47.71	16.829	0.241	6.751	0.578
MRS 3x SIRI # 26	48.8	56.75	48.62	14.302	0.248	10.374	0.531
MRS 3x SIRI # 29	48.6	58.75	47.82	17.821	0.217	6.084	0.495
MRS 3x SIRI # 33	45.9	55.50	47.48	12.817	0.284	9.297	0.599
MRS 3x SIRI # 39	48.7	57.60	47.96	15.614	0.810	7.700	0.776
MRS 3x SIRI # 45	45.4	57.84	48.14	12.918	0.732	8.768	0.750

Note: PR = Photosynthetic rate (µmol$CO^2 m^{-2} s^{-1}$) SC = Conductance (mmol$H_2O^{m-2} s^{-1}$) TR = Transpiration rate (mmol$H_2O^{m-2} s^{-1}$) Ci/Ca = Intercellular CO_2/Ambient CO_2

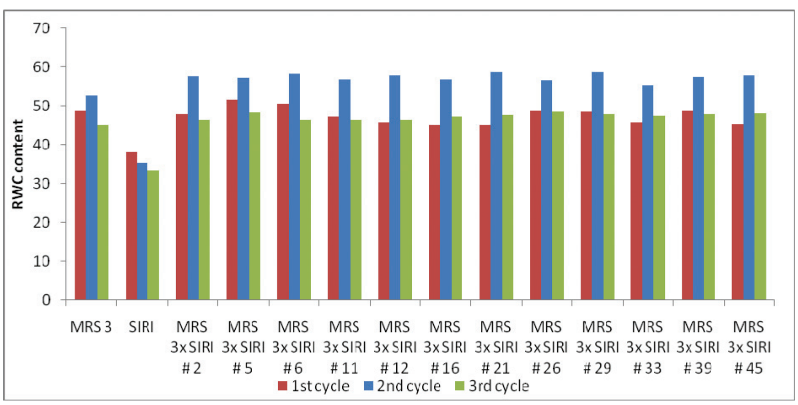

Fig. 3. RWC of drought tolerant genotypes under three cycles of intermittent water stress

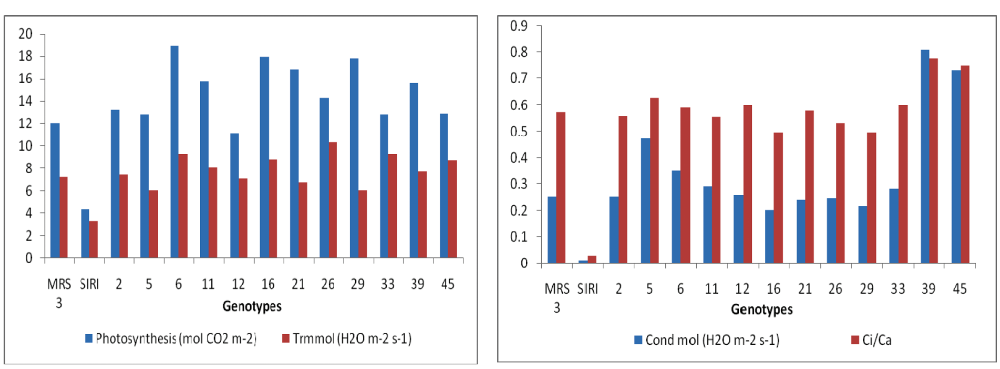

Fig.4. Physiology of drought tolerant genotypes after three cycles of intermittent water stress

Based on the morphological and physiological characters among the selected 12 genotypes, MRS 3x SIRI # 6, MRS 3x SIRI # 11, MRS 3x SIRI # 29 and MRS 3x SIRI # 39 were found to be superior. The selected genotypes are to be further evaluated at field condition to confirm their performance during water stress condition.

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