PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF SELECTED ORTETS AND HYBRIDS UNDER ABIOTIC STRESS CONDITIONS

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Sixteen ortets selected from different agro-climatic regions of India were tested for their initial performance along with seven check clones in the field under two extreme stress situations, *viz*. drought stress at Dapchari in Maharashtra and low temperature stress at Nagrakata in West Bengal. Under severe soil moisture deficit stress conditions, ortets RRSA 98, DAP 35 and GH 1 had better photochemical efficiency in terms of gas exchange and chlorophyll fluorescence. The photosynthetic activities declined sharply during low temperature stress (winter) at Nagrakata. Low temperature stress was found more detrimental to photosynthetic apparatus of young rubber plants than soil moisture deficit together with high temperature. Ortets, DAP 1, RRST 24, GH 1 and GH 9, showed relatively better CO_2 assimilation under low temperature stress. Ortets DAP 1, NGK 1 and GH 9 that had better CO_2 assimilation under low temperature condition did not perform equally well under drought conditions. The PS II activity of ortets and check clones did not vary significantly under drought conditions, however, it declined sharply in clones tested at Nagrakata during low temperature conditions, indicating the adverse effect of low temperature on photosystem II. Ortets GH 1, GH 9 and RRSA 585 showed better PS II activity during winter season. Soluble leaf protein content decreased in most of the plants tested under low temperature while it increased under drought. Malondialdehyde (MDA), a major lipid peroxidation product was less under low temperature conditions compared to drought in these plants. Among the ortets, GH 1 and GH 3 recorded comparatively high magnitude of reduction in sucrose content under stress in both the locations. Ortets GH 1, GH 3, DAP 1, NGK 1, DAP 35 and RRSA 98 recorded better adaptive traits under abiotic stress conditions in the early stages of growth and may be further shortlisted as potential clones suitable for extreme climatic conditions, which may also yield better, as their selection was primarily based on term performance of yield and other secondary traits in the fields.

Key words: Biochemical parameters, Cold stress, Drought, Ortet, Photosynthesis

INTRODUCTION

Natural rubber (*Hevea brasiliensis*) is cultivated in a wide range of agro-climate like the drought prone region in Maharashtra and Odisha, low temperature affected regions in North East India as well as in Bengal for the past few decades. Exposure to extreme stress conditions in these regions affects productivity of rubber plantations to a greater extent. Rubber clones that are high yielding in the traditional rubber growing region may not yield well in these

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extreme environments. Hence, high yielding rubber plants that are well adapted to these harsh environments need to be identified and multiplied for large scale cultivation of these clones in stress prone regions.

Drought and high temperature during summer and low temperature during winter are the major environmental factors that restrict further expansion of rubber plantation to newer areas. Regions of North East India suffer from extreme low temperature during winter and some semi-arid regions in Konkan coast and central India suffer from drought and high temperature every year. Prevalence of high light is a common feature in both these regions, which further aggravates the damage caused to the plants by these stresses (Devakumar *et al*., 2002).

Rubber Research Institute of India has Regional Research Stations located in some of these stress prone regions, where crop improvement programmes are being done for many years. These stations have identified their potential polyclones/ortets that show significantly better growth and yield, under the respective agro-climatic conditions. Ortet is a selected individual seedling tree based on yield and secondary characters, and further multiplied vegetatively for maintaining its superiority in these characters. It was also confirmed that these selections are performing better during immature as well as mature stages in terms of yield and other characters (Mydin *et al*., 2005, 2016; John *et al*., 2013; Mercykutty *et al*., 2013; Reju *et al*., 2016).

Natural rubber plants, grown in two distinct agro-climatic regions, showed severe inhibition in photosynthetic rate during unfavourable seasons, to the extent that the upper canopy leaves fixed very little carbon for most of the day (Devakumar *et al*., 2002). When the photosynthetic carbon assimilation rates are inhibited disproportionately, more than inhibition of photochemistry, molecular oxygen becomes an alternative sink for photosynthetic electrons producing large quantities of active oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals, which are otherwise effectively detoxified by various enzymatic and non-enzymatic antioxidant defence mechanisms (Jacob and Karaba, 2000). The antioxidant defence system in relation to drought and low temperature (Devakumar *et al*., 2002) and drought alone (Sreelatha *et al*., 2003) has been studied in *Hevea* indicating their role in scavenging the reactive oxygen species and protecting the cells from oxidative stress.

At cellular level, the mechanism of drought and cold tolerance in these clones may be similar or different. The performance of cold tolerant clones/ortets identified from North East region, when grown under drought/high temperature conditions may be the same or different making the plant susceptible or tolerant to the new situation. The objectives of the present study were to evaluate the photosynthetic and biochemical responses of selected ortets and check clones under stress and non-stress periods in the field under the hot and dry conditions at Dapchari in Maharshtra and very cold conditions at Nagrakata in Bengal compared to the non-stress situations in the traditional region at Central Experiment Station, Chethackal in Kerala.

MATERIALS AND METHODS

The experiments were carried out in field grown budgrafted ortets and check clones in three locations, *ie.*, Dapchari, Nagrakata and Chethackal during 2014 and 2015. Dapchari is situated in the North Konkan region of Maharashtra state, at 20°04' N, 72°04' E, 48 m above MSL, experiencing prolonged drought

and high temperature during January to June every year (Das *et al.*, 2016). The average rainfall of this area is 2400 mm received from June to December, and practically no rain for the rest of the year. Dapchari receives a high solar radiation load of 2000 µ molesm^{-2}s^{-1} on a typical summer day, high temperature (above 38^{o}C) and low atmospheric relative humidity (25 to 40 %) during peak summer. Nagrakata, located in the foot hills of Himalayas experiences cold winter from November to late February every year (Das *et al.*, 2016). During peak winter (early January), the minimum temperature can be as low as 5 ^{o}C for a few days. The annual rainfall in this region is about 2000 mm, which is well distributed round the year. Chethackal located in Central Kerala (9^{o}26' N and 76^{o}48' E) has a moderate climate (with average maximum and minimum temperatures of 33^{o}C and 22 ^{o}C, respectively and annual rainfall of about 3000 mm) compared to the other two locations and is selected as a control location to compare the drought and low temperature effect on the performance of these plants.

Sixteen ortets from five locations and seven check clones (hybrids) were selected for the study (Table 1). Planting of the field experiment was done during 2012, at all the three locations. Photosynthetic measurements were taken during the second and third year of planting. The measurements were taken during stress free periods at Dapchari (September), Nagrakata (September-October) and Chethackal (September), while observations of stress periods were taken during April-May in Dapchari (peak summer) and December-January in Nagrakata (peak winter). Irrigation was not provided to plants except in Dapchari, where life-saving irrigation @ 40 litres at 15 days interval was given during peak summer. Six plants of each ortet/clone with uniform growth were selected and measurements were taken on two leaves of each plant. Photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration (E) were measured using a portable photosynthesis system (LI-6400XT, Li-Cor, USA), with a reference CO_2 of 400 µmol (CO_2) mol(air)$^{-1}$ and light intensity 500 µmol m^{-2} s^{-1}. Measurements were done in intact, mature, fully expanded leaves by inserting the leaves into the chamber and logged when photosynthesis became stable (Alam *et al.*, 2005). The effective quantum yield of PS II ($\Phi_{PS\ II}$) was also simultaneoasly measured using the same instrument. The light adapted leaves of the top mature whorl were selected for measurements and after the fluorescence emission at a given light because at stable; a saturating pulse of light was given for getting light adapted quatum yield of PS II (Schreiber, 2004).

Leaf samples for biochemical analysis were taken during stress and stress free periods at Dapchari and Nagrakata and stress free period at Chethackal during

Table 1. **Ortets and check clones selected for the study and their origin**

Ortets/Clones	Selection from
DAP 1, DAP 34, DAP 35, DAP 36	RRS, Dapchari, Maharashtra
RRSA 98, RRSA 315, RRSA 585	RRS, Agartala, Tripura
NGK 1, NGK 47, NGK 69	RES, Nagrakatta, West Bengal
GH 1, GH 3, GH 9	RRS, Guwahati, Assam
RRST 24, RRST 37, RRST 39	RRS, Tura, Meghalaya
RRII 105, RRII 414, RRII 417, RRII 422, RRII 429, RRII 430, RRIM 600	Check clones

second year of planting. After making the gas exchange measurements, the leaves were excised and immediately frozen in liquid nitrogen and transported to the laboratory on dry ice and preserved at -50°C until used for analysis.

Leaf extract for assay of peroxidase activity was prepared by homogenising leaf samples in an extraction buffer containing potassium phosphate and polyvinyl pyrrolidone. The homogenates were centrifuged and supernatant was used for assay of peroxidase activity as described by Amako *et al.* (1994) and Karr and Mishra (1976) and expressed in relative units per mg fresh weight of leaf tissue. One enzyme unit is defined as the change in absorbance per hour caused by the enzyme.

Total sugars and phenols were extracted in 80 per cent alcohol and measured as described by Scott and Melvin (1953) and Swain and Hills (1959), respectively. Total glutathione from leaves was extracted in five per cent (w/v) trichloroacetic acid (TCA), centrifuged at 10,000 rpm for 20 min. at room temperature, TCA removed using diethyl ether and estimated according to Boyne and Ellman (1972). Protein content in the extract was measured according to Lowry *et al.* (1951). Lipid peroxidation was estimated by determining the malondialdehyde (MDA) content in the leaf according to Heath and Packer (1968). Leaf samples were homogenized in 0.1 per cent trichloroacetic acid (TCA) and the homogenate was centrifuged at 12,000 rpm for 20 min. at 4 °C. About 0.3 ml of supernatant was mixed with 1.2 ml of 0.5 per cent thiobarbituric acid prepared in 20 per cent TCA and incubated at 95 °C for one hour. After stopping the reaction in an ice bath, the optical density was read at 532 nm and 600 nm. After subtracting the non-specific absorbance at 600 nm, MDA concentration was calculated using the extinction coefficient 155 mM^{-1} cm^{-1}.

Data presented are mean of five to six measurements and were analysed statistically by analysis of variance using SPSS software.

RESULTS AND DISCUSSION

Under extreme drought situations in Dapchari, P_N was reduced sharply in most of the ortets and hybrid clones (Table 2). Reduction was less in ortets like DAP 35, RRSA 98 and GH 3 and more in DAP 1 and GH 9. Stomatal conductance (g_s) was higher in RRSA 98 under drought stress, which also showed least decline in transpiration rate. DAP 35 and GH 1 were other ortets that had better g_s and transpiration rate under drought stress. Reduction in light adapted quantum yield was moderate in most of the ortets and hybrid clones under drought unlike in cold conditions, DAP 35 maintained stable PS II activity even under drought without any reduction while other clones and ortets showed a slight reduction in PS II quantum yield. A good evaluation of the photosynthetic performance of stressed plants is obtained by analysing the chlorophyll fluorescence parameters together with gas exchange parameters (Maxwell and Johnson, 2000).

Total sugar content increased in all the clones under drought, in general, except in ortets like GH 3, GH 9 and DAP 36 (Table 3). The protein content increased in most of the plants under drought stress, in contrast to the observation that the protein content decreased in most of the plants under cold stress. Marked increase in protein content was observed in NGK 1 followed by RRST 39 and DAP 34. Devakumar *et al.* (2002) reported increase in protein content and decrease in soluble sugars during peak summer in mature trees compared to post monsoon season at Dapchari, while phenols and

Table 2. **Photosynthetic parameters of the ortets and check clones during stress and non-stress seasons at RRS, Dapchari**

Ortet/ Clone	P_N (µmol m^{-2} s^{-1})			g_s(mol m^{-2} s^{-1})			Φ_{PSII}			E (mmol m^{-2} s^{-1})		
	Stress free	Drought stress	% reduction	Stress free	Drought stress	% reduction	Stress free	Drought stress	% reduction	Stress free	Drought stress	% reduction
DAP 1	12.7	4.0	68	0.24	0.03	89	0.48	0.36	25	5.0	0.9	81
DAP 34	10.9	5.6	49	0.14	0.04	71	0.47	0.45	4	2.8	0.9	66
DAP 35	8.2	8.6	-4	0.14	0.12	17	0.41	0.48	-18	2.7	2.4	13
DAP 36	10.2	5.1	50	0.12	0.05	62	0.50	0.41	17	2.6	1.2	56
RRSA 98	11.0	9.6	13	0.14	0.12	13	0.46	0.45	3	2.2	2.3	-4
RRSA 315	8.5	4.6	46	0.07	0.03	53	0.46	0.42	7	1.8	0.8	56
RRSA 585	11.4	6.8	40	0.19	0.08	58	0.48	0.46	3	4.1	2.4	41
NGK 1	11.8	5.3	55	0.17	0.06	66	0.47	0.45	6	3.1	1.2	59
NGK 47	12.5	6.8	45	0.16	0.07	58	0.50	0.40	20	2.8	1.5	45
NGK 69	12.0	6.2	48	0.15	0.05	66	0.50	0.47	7	3.5	1.4	61
GH 1	11.8	8.0	32	0.17	0.07	58	0.48	0.47	1	2.5	1.6	36
GH 3	10.1	7.9	22	0.17	0.07	56	0.45	0.40	10	3.9	1.8	53
GH 9	12.0	2.7	77	0.17	0.03	82	0.49	0.33	32	4.5	0.9	80
RRST 24	9.3	4.8	48	0.11	0.04	64	0.45	0.39	14	2.8	1.0	64
RRST 37	12.0	5.9	51	0.21	0.07	68	0.48	0.46	4	4.0	2.2	44
RRST 39	12.8	7.3	43	0.19	0.07	63	0.51	0.50	3	4.4	1.8	59
RRII 105	12.5	7.5	40	0.22	0.07	66	0.47	0.48	-1	3.5	1.6	53
RRIM 600	13.7	3.9	71	0.22	0.04	83	0.47	0.41	13	3.7	1.6	58
RRII 414	9.3	5.5	41	0.13	0.04	70	0.45	0.44	3	2.1	1.0	53
RRII 417	8.8	4.9	44	0.08	0.05	43	0.45	0.39	14	1.6	1.0	40
RRII 422	9.6	8.5	12	0.11	0.08	24	0.48	0.46	3	2.7	2.0	24
RRII 429	10.9	4.7	57	0.12	0.04	62	0.45	0.40	12	2.1	1.2	44
RRII 430	11.4	6.8	40	0.15	0.09	41	0.47	0.36	24	2.1	1.7	20
CD (clone x envi.)		PN: 2.31			gs: 0.039			$\Phi_{PS\ II}$: 0.055			E: 0.71	

Table 3.**Biochemical parameters of the ortets and check clones during stress and non-stress season at RRS, Dapchari**

Ortet/ Clone	Total sugar (mg g^{-1}fw)			Protein (mg g^{-1}fw)			MDA (µmol g^{-1}fw)			Phenol (mg g^{-1}fw)			Px (units)			Glutathione (mg g^{-1}fw)		
	stress free	drought	% change	stress free	drought	% change	stress free	drought	% change	stress free	drought	% change	stress free	drought	% change	stress free	drought	% change
DAP 1	65.1	88.7	-36	48.4	38.9	20	12.8	17.5	-36	5.0	7.6	-51	1.5	0.9	42	7.6	7.9	-4
DAP 34	55.5	87.4	-58	25.5	48.1	-88	8.9	15.5	-75	6.2	6.0	4	2.3	1.1	54	9.0	5.5	38
DAP 35	50.2	70.4	-40	42.7	46.9	-10	25.5	14.5	43	7.3	5.7	22	1.6	0.8	48	9.5	6.4	33
DAP 36	58.0	54.9	5	38.0	50.3	-32	17.2	15.4	11	5.0	5.2	-5	1.0	1.0	-3	9.8	5.0	49
RRSA 98	47.0	70.7	-50	31.1	41.9	-35	12.4	12.1	2	6.5	7.1	-10	1.4	1.7	-16	8.5	5.5	35
RRSA 315	72.2	79.4	-10	33.4	38.0	-14	20.6	13.7	33	6.4	4.8	25	1.7	0.9	49	11.7	5.8	51
RRSA 585	55.2	77.1	-40	33.7	59.1	-75	19.1	13.8	28	6.1	6.9	-14	2.3	1.1	50	9.7	6.5	33
NGK 1	62.3	68.9	-11	12.1	35.1	-191	15.9	13.3	16	11.1	5.9	46	2.9	1.4	53	7.3	5.6	23
NGK 47	50.8	65.3	-28	38.8	57.4	-48	17.1	8.2	52	4.3	4.5	-4	1.5	1.0	36	7.9	4.7	41
NGK 69	49.7	37.0	26	40.7	51.0	-25	14.4	18.4	-28	7.2	4.8	33	1.5	1.2	21	10.4	8.5	18
GH 1	54.3	67.8	-25	31.0	40.4	-30	19.7	12.5	37	4.3	4.9	-15	3.0	1.4	53	8.0	5.3	33
GH 3	66.6	65.7	1	48.2	48.3	0	25.3	18.0	29	4.6	3.1	33	2.3	0.9	62	9.6	5.9	38
GH 9	53.7	54.9	-2	43.8	52.8	-21	18.8	9.8	48	5.4	5.9	-10	1.9	1.1	41	7.6	5.6	27
RRST 24	50.9	79.0	-55	60.2	46.0	23	13.0	10.6	18	2.9	5.9	-100	2.3	1.4	37	5.2	4.7	10
RRST 37	51.3	56.0	-9	23.9	42.6	-78	15.8	12.9	18	5.6	4.9	12	3.1	1.7	46	5.8	5.1	12
RRST 39	49.2	65.6	-33	20.4	39.1	-92	14.7	10.6	28	10.3	7.2	30	1.4	1.1	23	7.0	4.9	29
RRII 105	56.9	68.6	-21	60.0	41.7	31	27.4	11.5	58	4.4	4.2	6	1.7	2.5	-46	7.8	5.1	34
RRIM 600	67.9	51.7	24	36.7	28.8	22	21.2	12.0	43	4.9	5.4	-9	2.4	2.1	15	9.1	3.2	65
RRII 414	51.5	26.2	49	31.3	33.1	-6	11.6	15.3	-32	3.6	5.1	-41	2.8	2.4	13	6.4	4.1	36
RRII 417	47.7	52.5	-10	32.5	46.3	-42	11.3	15.9	-41	4.5	4.4	2	2.6	2.1	20	9.4	5.2	44
RRII 422	62.3	65.7	-5	20.5	51.6	-152	12.3	14.8	-21	3.7	4.7	-28	2.6	1.6	36	8.8	5.2	41
RRII 429	38.1	92.9	-144	23.6	46.4	-96	13.9	9.9	29	2.6	5.3	-106	1.8	1.7	1	6.3	6.7	-6
RRII 430	45.6	69.0	-51	37.3	58.6	-57	10.0	7.1	29	1.7	5.4	-211	1.6	1.0	39	6.8	5.9	13
CD(clone x envi.)		13.95			11.7			5.0			2.2			0.85			NS	

Table 4. **Photosynthetic parameters of the ortets and check clones during stress and non-stress season at RRS, Nagrakata**

Ortet/ Clone	P_N (µmol m^{-2} s^{-1})			g_s(mol m^{-2} s^{-1})			Φ_{PSII}			E (mmol m^{-2} s^{-1})		
	Stress free	Cold stress	Percentage reduction	Stress free	Cold stress	Percentage reduction	Stress free	Cold stress	Percentage reduction	Stress free	Cold stress	Percentage reduction
DAP 1	13.5	1.2	91	0.22	0.00	101	0.50	0.15	69	3.5	0.0	101
DAP 34	12.4	-0.3	102	0.32	0.01	96	0.53	0.11	79	3.7	0.1	96
DAP 35	13.6	0.8	94	0.31	0.01	98	0.49	0.14	72	5.0	0.0	99
DAP 36	14.5	0.1	99	0.34	0.02	95	0.46	0.14	70	4.3	0.2	95
RRSA 98	14.6	0.0	100	0.36	-0.02	104	0.50	0.08	83	4.2	-0.3	106
RRSA 315	11.5	0.1	99	0.29	0.00	101	0.50	0.08	83	5.2	0.0	101
RRSA 585	14.9	0.0	100	0.35	-0.01	104	0.52	0.14	72	4.3	-0.1	102
NGK 1	14.4	-1.5	111	0.34	0.06	83	0.52	0.09	83	3.6	0.7	80
NGK 47	12.6	-0.7	106	0.26	0.00	98	0.50	0.15	70	3.8	0.0	99
NGK 69	13.7	-1.5	111	0.32	-0.01	104	0.53	0.13	75	4.5	-0.2	104
GH 1	14.8	1.1	93	0.31	-0.02	106	0.48	0.18	62	4.5	-0.2	105
GH 3	14.9	-1.2	108	0.32	0.03	92	0.49	0.05	89	4.4	0.2	95
GH 9	13.7	1.0	92	0.23	-0.03	111	0.50	0.25	50	4.3	-0.2	106
RRST 24	14.3	0.4	97	0.27	0.02	93	0.55	0.13	76	3.9	0.2	95
RRST 37	14.6	-0.6	104	0.38	0.01	98	0.53	0.11	80	5.5	0.1	97
RRST 39	15.7	0.6	96	0.44	0.01	97	0.53	0.10	81	4.9	0.1	97
RRII 105	15.1	0.8	95	0.31	-0.01	103	0.54	0.20	62	4.2	-0.1	102
RRIM 600	14.6	0.7	95	0.36	0.03	92	0.54	0.10	82	4.5	0.5	88
RRII 414	15.1	0.0	100	0.30	0.05	83	0.51	0.07	87	4.3	0.9	79
RRII 417	14.7	2.3	84	0.31	0.02	93	0.51	0.15	71	4.4	0.3	93
RRII 422	14.2	1.5	90	0.32	0.02	95	0.51	0.16	68	4.2	0.3	93
RRII 429	13.6	2.7	80	0.30	0.02	95	0.54	0.28	48	3.7	0.3	92
RRII 430	14.7	0.8	94	0.29	0.01	95	0.50	0.23	54	2.9	0.2	93
CD (Clone x envi.)		P_N: 1.27			gs: 0.068			Φ_{PSII} 0.039			0.72	

Table 5. **Biochemical parameters of the ortets and check clones during stress and non-stress season at RRS, Nagrakata**

Clone	Total sugar (mg g^{-1}fw)			Protein (mg g^{-1}fw)			MDA (µmol g^{-1}fw)			Phenol (mg g^{-1}fw)			Px (units)			Glutathione (mg g^{-1}fw)		
	Stress free	Cold stress	% change	Stress free	Cold stress	% change	Stress free	Cold stress	% change	Stress free	Cold stress	% change	Stress free	Cold stress	% change	Stress free	Cold stress	% change
DAP 1	54.9	77.9	-42	26.9	20.8	23	21.1	5.51	74	13.0	7.6	42	0.6	0.4	34	6.3	6.5	-3
DAP 34	38.0	69.0	-81	23.6	20.8	12	16.7	6.74	60	6.3	8.3	-31	1.1	0.8	25	9.5	6.4	33
DAP 35	59.4	56.3	5	46.4	18.5	60	26.8	10.06	63	4.3	5.5	-28	1.8	2.3	-32	8.9	7.1	21
DAP 36	57.8	93.1	-61	21.6	24.5	-13	31.7	10.76	66	9.8	6.4	35	1.6	1.8	-11	9.2	8.3	9
RRSA 98	51.6	67.6	-31	30.2	21.4	29	32.4	5.50	83	2.8	9.5	-242	1.8	0.6	69	8.9	6.4	29
RRSA 315	67.2	65.5	3	33.6	16.7	50	18.6	7.38	60	6.4	9.1	-41	1.8	0.5	70	8.3	7.8	6
RRSA 585	52.5	78.8	-50	29.0	19.0	34	19.7	7.86	60	2.0	7.5	-281	2.9	1.9	35	5.8	6.8	-17
NGK 1	65.3	98.6	-51	23.4	18.1	23	18.2	6.15	66	6.0	10.4	-73	2.3	2.1	11	6.7	5.8	14
NGK 47	73.7	61.2	17	33.7	23.8	29	21.3	6.24	71	13.6	6.8	50	0.6	1.4	-151	9.0	5.9	34
NGK 69	66.8	59.3	11	25.1	18.4	26	20.1	6.22	69	2.7	6.5	-142	1.9	2.0	-3	7.3	5.3	27
GH 1	68.7	55.0	20	32.2	21.9	32	26.6	7.58	72	3.5	5.5	-60	1.4	1.3	9	7.6	7.3	4
GH 3	86.3	68.7	20	23.2	12.3	47	27.6	7.26	74	6.3	8.3	-31	3.0	0.6	82	5.4	4.4	19
GH 9	66.0	63.1	4	29.7	19.0	36	23.3	5.74	75	9.1	7.6	16	2.4	2.2	9	6.6	5.9	11
RRST 24	52.3	63.9	-22	29.9	15.8	47	18.5	6.83	63	4.2	7.8	-87	1.5	0.7	51	7.5	7.1	5
RRST 37	68.4	50.6	26	28.5	15.8	45	19.7	4.56	77	10.7	9.9	7	0.6	1.8	-217	7.5	4.9	34
RRST 39	66.9	70.7	-6	32.7	12.5	62	21.4	4.05	81	2.3	5.0	-114	1.5	2.5	-67	6.9	5.4	21
RRII 105	37.4	60.1	-61	21.6	16.8	22	19.5	5.60	71	5.0	6.7	-36	1.5	1.1	28	6.4	5.5	14
RRIM 600	53.8	70.3	-31	27.6	10.7	61	16.1	4.97	69	7.1	8.6	-21	1.2	1.0	11	7.5	7.5	0
RRII 414	33.7	69.8	-107	22.0	9.9	55	23.5	5.03	79	6.0	8.2	-36	1.4	1.6	-14	8.5	4.9	42
RRII 417	68.6	55.2	19	27.4	9.2	67	19.6	4.24	78	3.0	12.5	-323	2.3	0.3	88	7.6	5.9	23
RRII 422	67.8	47.9	29	24.9	13.7	45	12.6	3.53	72	4.7	21.4	-354	2.8	0.4	85	7.0	4.4	37
RRII 429	81.6	61.2	25	23.8	20.2	15	8.9	6.01	32	7.3	5.5	24	1.7	1.7	3	8.3	6.9	17
RRII 430	57.5	48.1	16	28.6	11.2	61	21.4	6.85	68	3.4	8.8	-160	2.9	1.5	47	8.8	6.6	25
CD (Clone x envi.)		9.7			7.6			4.0			2.4			0.75			1.7	

Table 6. **Photosynthetic and biochemical parameters of ortets and check clones during non-stress situation at CES,Chethackal**

Ortet/ clone	P_N ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	Φ_{PSII}	E ($mmol\ m^{-2}\ s^{-1}$)	Total sugar ($mg\ g^{-1}$ fw)	Protein ($mg\ g^{-1}$ fw)	MDA ($\mu mol\ g^{-1}$ fw)	Phenol ($mg\ g^{-1}$ fw)	Px (units)	Glutathione ($mg\ g^{-1}$ fw)
DAP1	11.4	0.26	0.47	3.9	27.5	11.2	15.7	10.4	2.9	2.2
DAP34	11.8	0.28	0.49	4.5	77.0	24.8	12.7	11.1	1.1	7.6
DAP35	13.3	0.31	0.50	4.3	70.8	16.7	14.1	6.3	1.6	6.7
DAP36	12.6	0.26	0.48	3.3	72.1	15.4	13.2	4.9	1.3	6.8
RRSA98	13.8	0.32	0.49	4.7	72.4	16.9	9.1	7.5	2.2	5.8
RRSA315	11.2	0.22	0.51	3.5	52.7	14.1	10.2	8.1	1.3	6.1
RRSA585	14.7	0.35	0.50	4.2	64.2	16.5	10.3	6.5	1.4	5.4
NGK1	15.4	0.41	0.51	3.8	80.9	10.7	10.2	6.0	2.6	5.7
NGK47	11.2	0.22	0.45	3.3	61.6	11.3	12.0	6.3	3.1	5.3
NGK69	11.8	0.27	0.48	4.2	90.5	20.1	16.1	5.1	1.3	7.8
GH1	13.0	0.31	0.48	4.9	68.6	16.4	11.1	5.5	2.1	5.8
GH3	13.6	0.33	0.50	4.4	75.1	17.8	13.5	10.6	2.2	5.1
GH9	14.4	0.32	0.48	3.9	71.5	20.0	11.2	6.4	1.7	6.7
RRST 24	13.7	0.30	0.49	3.7	55.8	21.4	10.4	9.1	2.7	6.7
RRST 37	14.2	0.34	0.54	4.1	75.8	17.1	11.7	7.3	1.6	6.6
RRST 39	13.9	0.36	0.49	4.4	73.1	16.7	12.2	10.5	2.0	6.5
RRII105	13.8	0.32	0.53	3.7	69.3	16.2	9.3	11.4	2.2	6.0
RRIM600	12.7	0.30	0.53	4.9	60.2	21.4	10.9	6.8	1.9	6.6
RRII414	12.0	0.32	0.49	3.5	83.6	13.3	13.0	8.5	2.4	6.5
RRII417	12.8	0.29	0.48	3.8	64.5	12.9	6.8	7.9	1.3	7.3
RRII422	12.1	0.29	0.51	3.6	66.6	11.7	5.2	5.6	1.0	5.7
RRII429	12.2	0.29	0.50	3.9	84.7	16.8	11.9	10.7	1.2	7.2
RRII430	14.4	0.28	0.47	3.5	61.3	13.5	10.4	9.1	1.0	6.5
CD(P=0.05)	1.8	0.07	0.04	0.7	11.1	3.7	3.3	2.6	0.6	1.6

glutathione did not show much variation during these periods.

MDA content increased in ortets DAP 34, DAP 1 and NGK 69 while it reduced in all other clones with more reduction (nearly 50%) in GH 9 and NGK 47. Phenol content almost doubled in RRST 24 under drought. Peroxidase activity showed a decline under drought condition, while RRSA 98 and DAP 36 had little variations from stress free season. In an earlier study, the levels of biochemical components such as proteins, phenols, glutathiones and sugars of the leaves of high and low yielding trees did not show appreciable variation during stress and stress free seasons at Dapchari (Sreelatha *et al.,* 2003). They have established significant association of higher leaf peroxidase activity with drought tolerance potential in *Hevea*, though, in the present study no major increase in peroxidase activity was observed in majority of plants tested (Tabel 3).

Under extreme cold conditions at Nagrakata, P_N of all the plants reduced drastically to near zero and even lesser in some ortets (Table 4). DAP 1, GH 1 and GH 3 had assimilation rates above 1 $\mu molm^{-2}s^{-1}$, indicating these ortets are having relatively better tolerance in terms of better photochemical efficiency compared to other ortets or check clones. Stomatal conductance also declined in all the plants, as a result of closure of stomata, which also prevented transpiration under the prevailing situation. It was seen that the plants that had relatively less decline in P_N like DAP 1, GH 1 and GH 3 had marked decline in stomatal conductance and transpiration rates. GH 1, GH 9 and DAP 1 showed relatively lesser reduction in effective quantum yield of PS II compared to stress free situations. Cold stress had profound effect on NGK 1, GH 3 and RRSA 98 which showed much higher reduction in photosynthetic activity among the ortets/ clones tested.

Photosynthetic process is often the first to be inhibited at low temperatures. In tree crops, when temperature drops below 15 ^oC, the major components of photosynthetic process including CO_2 diffusion through stomata, electron transport across photosystems and carbon reduction will be disrupted drastically (Mai *et al.,* 2010).

Total sugar content under cold stress showed significant variability among the ortets and hybrid clones tested. DAP 34 and DAP 35 which are selections from drought prone region showed an increase in sucrose content while GH 1, GH 3 and RRST 37 showed a decline in sucrose content by 20 to 26 per cent on exposure to severe cold (Table 5). Protein content declined in all the ortets and hybrid clones under cold stress. The decline was more in DAP 35, RRSA 315 and RRST 39 while DAP 1, DAP 34 and DAP 36 the decline in protein content was less. Devakumar *et al*. (2002) reported a decline in leaf protein content and increased concentrations of soluble sugars and glutathione during winter at Nagrakata.

Malondialdehyde content reduced in all the ortets and hybrid clones by 60 to 80 per cent during winter (Table 5). Phenol content and peroxidase activity showed varied response under cold stress conditions. Das *et al.* (2016) reported DAP 1 to be a highly potential cold tolerant and RRSA 98 as a highly susceptible ortet to low temperature stress which also support the findings of the present study.

Chethackal in Kerala, which falls in the traditional region was taken as a control region for comparing the performance of ortets at Dapcahri and Nagrakata (Table 6). The CO_2 assimilation of two year old plants in the field at Chethackal recorded rates as high as 11.2 to 15.4 $\mu mol\ m^{-2}\ s^{-1}$. Maximum

rate of assimilation was seen in NGK 1, followed by RRSA 585, GH 9, and RRII 430, while it was less in RRSA 315 and NGK 47. Stomatal conductance also showed similar trend in these selections. But, transpiration was highest in RRIM 600, GH 1 and RRSA 98. There was not much variation in $\Phi_{PS\ II}$ between these plants, and for most of the clones/ ortets it was around 0.5. Total sugar content was almost in the same range in the three regions under stress free conditions, except DAP 1 which showed much lower concentration at Chethackal (27.5µmol m^{-2} s^{-1}). Under stress free season, plants in Dapchari had higher protein content, followed by Nagrakata and CES, Chethackal. Malondialdehyde content was higher under stress free conditions in Nagrakata relative to the other two regions. Peroxidase and phenol content was similar in all the three regions with a few exceptions.

CONCLUSION

Evaluation of sixteen ortets and seven hybrid clones showed that the same plants will perform in a different pattern with respect to tolerance to abiotic stresses, when grown in a different agro-climatic cinditions. Ortets like GH 1 and GH 3 showed better adaptability under drought as well as cold situations, while ortets DAP 1 and NGK 1 performed better under cold, DAP 35 and RRSA 98 performed better under drought, in terms of the parameters tested.

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