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LEAF POTASSIUM CONTENT AS AN INDEX OF ADAPTATION TO DROUGHT TOLERANCE IN NATURAL RUBBER

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To find out whether potassium accumulation is more in drought tolerant accessions/clones of rubber (*Hevea brasiliensis*) and whether its concentration is high in accessions/clones during stress period, studies were conducted in selected clones and germplasm accessions. In the first experiment, leaf samples from mature trees were collected from a clone evaluation trial in the traditional rubber growing region of Kerala. In the second experiment, leaf samples of young natural rubber plants from already identified drought tolerant and susceptible wild accessions and hybrid clones grown in a drought prone area were assessed during pre-stress, stress and post-stress period. Significant differences in K content in leaves were recorded between genotypes in both experiments. RRII 430, a known drought tolerant clone recorded high K content in the leaves in both experiments. There was significantly higher K content in leaves in drought tolerant germplasm accessions than susceptible accessions. The leaf K concentration was high during stress compared to pre-stress and post-stress period in almost all accessions/clones. Five accessions/clones *viz.* MT 4788, MT 43, RO 2153, RRII 430, RRII 414 were identified as potential drought tolerant clones based on K content of leaves. High leaf K concentration in proven drought tolerant wild accessions/clones and also during drought stress, indicates high leaf K as an indication of adaptation to drought stress in *Hevea*. Hence, high leaf K concentration can be used as a screening tool for drought tolerance along with other physiological/ biochemical/parameters molecular.

Key words: Drought tolerance, *Hevea brasiliensis*, Clones/accessions, Leaf potassium concentration

INTRODUCTION

Drought tolerance is a key trait for increasing and stabilizing productivity in dry areas worldwide. Increasing intensity and frequency of dry period in many parts of the world due to global warming and changing climate make the agriculture production of any crop more challenging (Huang *et al.*, 2014). Evolving drought tolerant and climate resilient varieties is important for land and resource management to sustain productivity in these areas. Drought tolerance in plants is mainly through osmoregulation, potassium (K) accumulation for stomatal movement and protective role of K^+ in plants under drought stress has been well documented (Wang and Guo, 2013). Osmoregulation or osmotic adjustments refers to active accumulation of

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solutes in the cells during the period of water stress in which the important inorganic solute is K (Hosseini *et al.*, 2016). High K content in shoot of turf grass cultivars, wheat leaves, poplar leaves *etc.* during drought stress was reported (Marron *et al.*, 2002). Variation in K content with seasons (Gunes *et al.*, 2006) and high leaf K during drought stress in sugar cane (Silva *et al.*, 2017) was also reported.

Stressful environment caused by conditions such as drought is one of the important productivity limiting factors for rubber particularly in non-traditional areas and in parts of the traditional region. Drought tolerant and drought susceptible clones were identified earlier based on physiological parameter such as high peroxidase activity (Jacob and Karaba, 2000) and biochemical parameters such as APOX (ascorbate peroxidase) in the leaf and low PPO (Polyphenol oxidase) activity in bark (Sreelatha *et al.*, 2003) in *Hevea* clones. Young plants of RRII 430 was evaluated and screened as drought tolerant in Dapchari, Maharashtra, a drought stressed area in the non-traditional region (Ravichandran *et al.*, 2011). In the traditional area, RRII 430 was identified as drought tolerant by physiological parameters (Sumesh *et al.*, 2011), and through gene expression (Thomas *et al.*, 2011). Since the rubber growing areas are spread over areas prone to drought, cold and moisture stress, the present study finds relevance, where the leaf K content of drought tolerant and susceptible wild accessions/clones were assessed in the traditional and drought stressed non-traditional region.

MATERIALS AND METHODS

Concentration of K in the leaves of wild accessions/hybrid clones were determined from two different experiments. In the first experiment, the location was the experimental farm of Rubber Research Institute of India (9^{0}32′N 76^{0}36′E, 73 M above MSL), Rubber Board, Kottayam, Kerala, the traditional region of rubber cultivation in India. The mean temperature prevailing is 27.4 ^{o}C and the area receives around 3095 mm rainfall in two major seasons during June-July and August-September along with summer rains during February. The leaf samples from mature trees of 23 years age from a small scale clone evaluation trial at the research farm of Rubber Research Institute of India were collected from RRII 414, RRII 430, RRII 422, RRII 417 and RRII 429 along with high yielding popular clone RRII 105 laid out in randomized block design with three replications with a plot size of six trees planted at with a spacing of 4.9m×4.9m. Leaf samples from the lower branches without direct sunlight as per recommended practice were collected from six trees of each clone before noon during February.

In the second experiment (young plants), leaf samples were collected from a drought evaluation study of the wild germplasm accessions and hybrid clones along with Wickham check clones at the Regional Research Station, Dapchari, Maharashtra, a drought prone area. The location is specific with high temperature exceeding 40-50 ^{o}C in April-May and high light intensity with soil moisture stress in summer months (Mercy *et al.*, 2011) with a peak during May after a continuous period of five months without rainfall from December-May. Leaf samples were collected from four basal leaves in the topmost whorls according to the recommended practice for young un-branched plants Karthikakuttyamma *et al.*, (2000). Four plants in each replication were selected to collect a composite sample in three seasons *viz.* during pre-stress (February), stress (May) and post-stress (September) period according to the classification of the area during 2011. The design was RBD with three replication and leaf samples were collected from three year old plants before noon. The accessions/ clones were wild Amazonian accessions *viz.* Mato Grosso (MT) and Rondonia (RO) and hybrid clones of Wickham origin. A total of 33 *Hevea* clones/accessions and hybrid clones were taken for the study. It includes the drought tolerant MT accessions (17 nos.) and RO accessions (5 nos.), hybrid clones (5 nos.) and proven drought tolerant check clones (4 nos.) along with a relatively drought susceptible check clone RRII 105 and the known susceptible clone Tjir 1 (Mercy *et al.*, 2011).

The leaf samples of both experiments were oven-dried at 65 °C, powdered and dried to 105 °C for constant weight and desiccated before taken for chemical analysis. The concentration of K was estimated as per Piper (1942).

The data on leaf K were statistically analysed by analysis of variance (ANOVA) to compare the clones in the first experiment. In the second experiment, the accessions/clones *vs* stress interaction effect were analysed by two factor ANOVA to compare the accessions/ clones in each season and between seasons. Leaf K content of accessions/clones over seasons were compared by ANOVA with mean values of all three seasons (Panse and Sukatme, 1961).

When the accessions/clones interaction effect were significant, further analysis were done using scatter plot. To construct the scatter diagram and grouping of the accessions/ clones based on K content, three scatter diagram (using two seasons in each diagram) with bi-plot by four quadrant were prepared. The axis of the bi-plot was the mean values of all accessions/clones in each season and based on this the accessions/ clones were placed into four quadrants and accordingly into four groups *viz.* Group 1 (high K in both seasons), Group 4 (low K in both seasons), Group 2 (high K in one season and low K in the other season) Group 3 (low K in one season and high K in the other season). In the diagram the serial number of accessions/clones according to Table 4 was given to indicate the accessions/clones in four groups. The commonly observed accessions/clones in Group 1 in all three diagrams were identified as most potential drought tolerant clones and the accessions/ cones in Group 4 as least tolerant / susceptible. The accessions/clones commonly observed in Group 1 and Group 4 were highlighted in the diagram.

RESULTS AND DISCUSSION

The leaf K concentration of mature trees of RRII 400 series clones are given in Table 1. Among the clones, highest leaf K was observed in RRII 430. All RRII 400 series clones had high K content than the relatively susceptible clone RRII 105.

RRII 400 series clones were developed from a cross between RRII 105 x RRIC 100. Generally all RRIC 100 series clones identified as drought tolerant were superior in growth and high leaf and bark K under stressed conditions (Samarappuli *et al.*, 1992)

Table 1. **Concentration of potassium in the leaves of different clones in experiment 1**

Clone	Leaf potassium (%)
RRII 430	1.67
RRII 414	1.19
RRII 417	0.96
RRII 429	0.89
RRII 422	0.85
RRII 105	0.66
CD (P=0.05)	0.07

Table 2. **Concentration of potassium (%) in the leaves during three seasons in experiment II**

Sl. No.	Accessions	Pre stress (February)	Stress (May)	Post stress (September)	Mean (over seasons)
MT accessions					
1	MT 1627	0.92	1.93	0.97	1.27
2	MT 67	1.09	0.93	0.89	0.97
3	MT 1668	0.91	1.35	0.93	1.06
4	MT 1649	1.41	1.55	0.94	1.30
5	MT 41	0.84	0.88	0.79	0.84
6	MT 1616	0.89	1.07	0.99	0.98
7	MT 5100	1.28	1.69	0.86	1.28
8	MT 1619	0.85	1.11	0.76	0.90
9	MT 5078	1.06	0.78	0.94	0.92
10	MT 54	1.17	1.41	0.99	1.19
11	MT 4788	1.34	1.69	1.17	1.40
12	MT 1623	0.86	1.43	1.19	1.16
13	MT 4856	0.86	1.07	0.99	0.97
14	MT 1681	1.34	1.42	0.92	1.22
15	MT 1710	0.85	1.16	1.13	1.05
16	MT 43	1.26	1.42	1.32	1.33
17	MT 40	0.92	1.08	0.76	0.92
18	RO 2153	1.23	1.44	1.28	1.32
19	RO 1761	0.92	1.37	1.05	1.11
20	RO 1769	0.87	1.11	0.99	0.99
21	RO 2387	0.79	0.99	0.96	0.91
22	RO 85	0.99	1.09	1.13	1.07
Hybrid clones					
23	93/ 270	1.24	1.14	1.26	1.21
24	93/ 105	1.07	1.18	1.15	1.13
25	93/ 92	1.48	1.52	0.91	1.30
26	93/ 225	1.23	1.56	0.91	1.23
27	93/ 53	1.16	0.89	0.92	0.99
Drought tolerant clones					
28	RRII 430	1.27	1.73	1.24	1.41
29	RRII 414	1.18	1.33	1.11	1.21
30	RRII 208	1.14	1.25	1.11	1.16
31	RRIM 600	0.81	0.96	0.96	0.91
Relatively susceptible clone					
32	RRII 105	1.15	1.31	1.09	1.18
Susceptible clone					
33	Tjir 1	0.74	0.51	0.72	0.66
Range		0.74-1.48	0.51-1.93	0.72-1.28	
Mean		1.06	1.25	1.01	
CD (P=0.05)		0.09			
CD(P=0.05) - (Accessions x season interaction)			0.15		

indicating its drought tolerance. Among the RRII 400 series clones, RRII 430, RRII 414 and RRII 417 recorded higher K content and RRII 429 and RRII 422 were on par. The clone RRII 430 was also performing well in the Padiyoor area in northern Kerala which is a relatively drought affected area in the traditional rubber growing region (Meenakumari *et al.*, 2015). Better CO_2 assimilation and photochemical efficiency in RRII 430 under drought stress and moisture stress indicated that RRII 430 was a potential clone to drought stress management in the changing climatic conditions (Sumesh *et al.*, 2011). Based on the juvenile performance in the drought prone Dapchari, Maharashtra, the clone RRII 430 was observed as a suitable clone for the drought stressed areas (Mercy *et al.*, 2011). Ravichandran *et al.* (2011) observed that the drying and leaf fall of the poly bag plants and field plants were less for RRII 430 in Dapchari. Physiological investigations, molecular gene expression studies and performances in the field in drought conditions already conducted in *Hevea* also revealed that this clone has drought tolerance property (Annamalainathan *et al.*, 2010; Thomas *et al.*, 2011). Since the high leaf K was observed in the clone proven for drought tolerance, the observation of high leaf K supports that it is an adaptation to drought tolerance.

Significant difference in leaf K content was observed in accessions/clones and among different seasons (Table 2). Leaf K content ranged from 0.74 - 1.48 (pre-stress), 0.51-1.93 (stress) and 0.72-1.28 (post-stress) with a mean value of 1.06, 1.25 and 1.01 per cent, respectively. In the pre-stress season, K content recorded in drought tolerant accessions were 1.48 per cent (93/92), 1.41 per cent (MT 1649), 1.34 per cent (MT 4788) and 1.34 per cent (MT 1681) followed by 1.28 per cent (MT 5100), 1.26 per cent (MT 43), 1.23 per cent (RO 2153), 1.24 per cent (93/270), 1.25 per cent (93/225) and 1.27 per cent (RRII 430).

During drought stress period, almost all clones/accessions showed increase in K-content compared to pre-stress and post stress period. Very high K content during drought stress was reported as an adaptive strategy (Muller *et al.*, 2017). The observed high leaf K concentration during stress may be the adaptive mechanisms to drought stress of rubber clones/accessions. Highest K content was observed in MT 1627 (1.93 %) followed by RRII 430 (1.73 %), MT 5100 (1.69 %) and MT 4788 (1.69 %). Accessions MT 1649 and hybrid clones 93/92, 93/225 were on par with K content in the range (1.5-1.56). Accessions MT 54, MT 1623, MT 1681, MT 43, RO 2153 with K content between (1.41-1.44 %) and check clones RRII 414 (1.33) and RRII 105 (1.31) were also on par. Nair *et al.* (2011) has reported low cell membrane injury and chlorophyll bleaching combined with reduced PS II activity in accessions MT 5100 and MT 4788. These two accessions showed higher K content during stress in the present study which is also an indication of high K as an adaptation to drought tolerance. Mercy *et al.* (2010) observed that, the drought tolerance potential was higher in Mato Grosso and Rondonia accessions than Acre accessions under field evaluation in Dapchari, Maharashtra. Very low K content was observed in the known susceptible clone Tjir 1 compared to all accessions/clones during stress period indicating susceptibility to drought stress.

In post-stress season, almost all clones had only low K content in the range of 0.74-1.17 per cent. However, the accessions MT 43, RO 2153, 93/270 and clone RRII 430 had higher K content of 1.32, 1.28, 1.26 and

1.24 per cent, respectively than other clones in post-stress season. These accessions/ clones showed high K content in stress period also where MT 1627 and RRII 430 were ranked with higher K content.

The drought tolerant accessions/clones recorded high K during pre-stress and stress were MT 1649, MT 5100, MT 54, MT 4788, MT 1681, MT 43, RO 2153, 93/92, 93/225, RRII 430, RRII 414 and RRII 105. Kant and Kafkafi (2002) reported that if the K content is in the luxury consumption category, the high K accumulation in pre-stress is a precautionary strategy for the survival to overcome the environmental stress like drought. The observation of higher K in pre-stress in accessions/clones of the present study may also indicate the precaution to overcome drought stress.

The higher K accumulation in pre-stress and during drought stress might be an adaptive mechanism for drought tolerance in rubber. Plants in their natural habitats adapt to drought through different mechanism that enable them to escape, avoid or tolerate drought (Basu *et al.*, 2016). High K in pre-stress and stress and the requirement of higher leaf K to tide over drought was reported in other crops (Muller *et al.*, 2017; Gunes *et al.*, 2006). Cakmak (2005) reported that plants suffering from drought stress required more internal K. Prasannakumari *et al.* (2011) reported that higher dose of K (2.5 times of the recommended dose) applied for young plants had a positive effect in overcoming the moisture stress with better leaf water potential and chlorophyll content. During drought condition, Reactive Oxygen Species (ROS) formation was induced and oxidative damage to cells occurred and requirement for K was increased (Foyer *et al.*, 2002), thus enhancing the need for K by plants

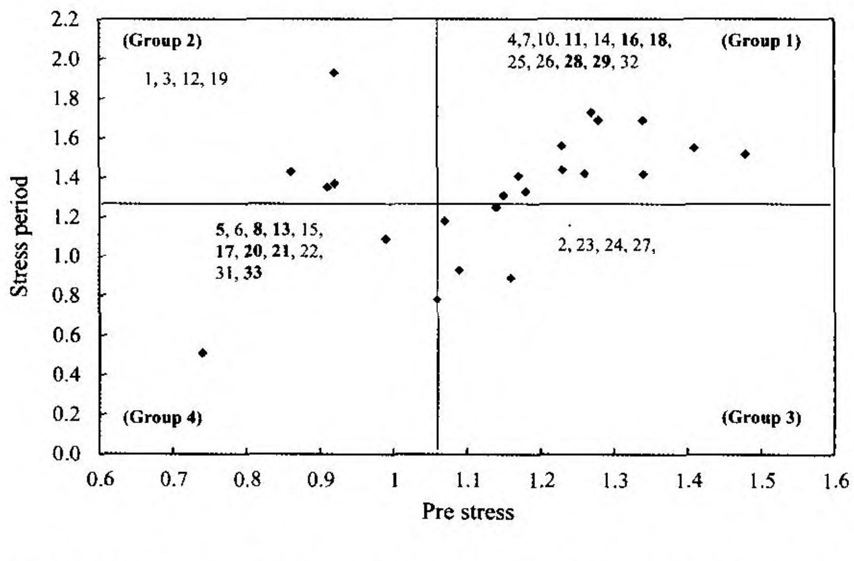

Fig. 1. Leaf K concentration (%) of accessions/clones grouped during pre-stress *vs* stress period

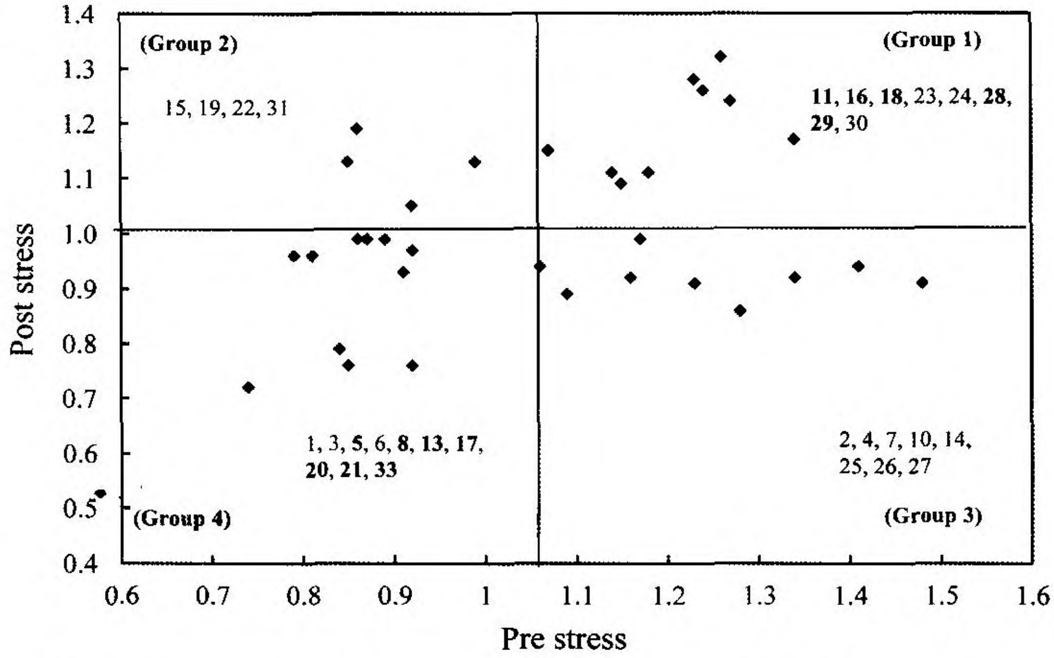

Fig. 2. Leaf K concentration (%) of accessions/clones grouped during pre-stress *vs post* - stress period

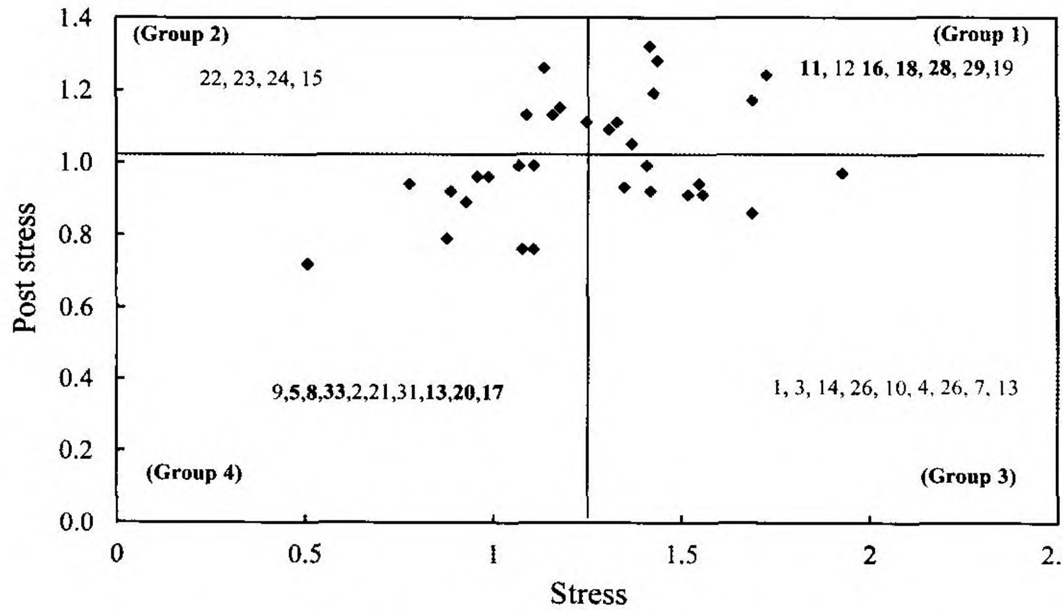

Fig. 3. Leaf K concentration (%) of accessions/clones grouped during stress *vs post*-stress period

suffering from drought stress. Various adaptive (resistant) mechanisms in the plant have been developed under stress conditions. According to Srinivasarao *et al.* (2009) mineral nutrient status of plants has major role in its adaptations to stress and reported enhanced leaf K content during drought in different species including trees.

The drought tolerant and susceptible accessions/clones were grouped by scatter diagram (Fig. 1-3) with bi plot constructed based on the variation in leaf K content in different seasons to cluster the accessions/ clones. The pre-stress period mean K value 1.06, the stress period mean K value 1.25 and post-stress mean K value 1.01 were used to generate the axes of the diagram and the leaf K value of each clone was placed into four quadrant. The accessions/clones with higher K content appeared in Group 1 in all the three diagrams and the common accession/ clones in all the three clustering diagram were MT 4788 (11), MT 43 (16), RO 2153 (18), RRII 430 (28) and RRII 414 (29) indicating their drought tolerance by way of higher K content (Table 3).

Similarly among the Group 4 accessions/ clones in all three clustering diagram, the accessions/clones MT 41 (5), MT 1619 (8), MT 4856 (13), RO 1710 (15) MT 40 (17), RO 1769 (20), RO 2387 (21) and Tjir 1 (33) were having lowest K indicating that they were least drought tolerant in terms of K.

Table 3. **Grouping of clones/accessions on the basis of drought tolerance /susceptibility**

Potential drought tolerant (Group 1)	Least drought tolerant/ susceptible (Group 4)
MT 4788 (11)	MT 41 (5)
MT43 (16)	MT 1619 (8)
RO 2153 (18)	MT 4856 (13)
RRII 430 (28)	MT 1710 (15)
RRII 414 (29)	MT 40 (17)
-	RO1769 (20)
-	RO 2387 (21)
-	Tjir 1 (33)

Sl. No. of the accessions/clones were given in parenthesis

The drought tolerance potential of the clones RRII 430 (28) and RRII 414 (29) was already identified by Mercy *et al.* (2010) and Thomas *et al.* (2011). The high K content of these two clones in all three seasons is also a proof of the relation of higher leaf K content to drought tolerance and the susceptible check clone Tjir 1 showed low K content in all three seasons indicating the relation between leaf K and drought tolerance in *Hevea*. Similar pattern of classification and grouping of clones based on the mean values were conducted in *Eucalyptus* clones under water stress (Muller *et al.*, 2017) and Sunflower (*Helianthus annuus* L.) for a germplasm evaluation for drought tolerance (Rauf *et al.*, 2007). High K accumulation in two seasons *viz.* early drought stress and late drought stress in drought tolerant cultivars of Chickpea (*Cicer arietinum* L.) was reported (Gunes *et al.*, 2006). Similar observation of high K content in pre stress and post stress period in some accessions/clones were observed in our study also.

High K uptake related to drought stress was reported in different crops and there were differences in K accumulation in stems, leaves, flowers and fruits (Leonard, 1985). Karyudi (2004) reported K is a major contributor to solute accumulation for rubber clones with high osmoregulation during water stress and had identified some clones based on this property as drought tolerant clones. In barley cultivars it was reported that, genotypes with high K nutritional status in the flag leaf show superior drought tolerance by promoting

ABA degradation but attenuating starch degradation which delays flag leaf senescence. Thus flag leaf K levels may thus represent a useful trait for the selection of drought tolerant barley cultivars (Hosseini *et al.*, 2016). Potassium channel protein and gene expressions related to higher K accumulation was reported in other crops (Britto and Kronzucker, 2008). Hu and Schmidhalter (2005) reported that drought can affect nutrient uptake and thereby the accumulation of K. Wang *et al.* (2013) reported the effect of K on drought tolerant mechanisms and major role of K in relation to various biotic and abiotic stress. Observations in the present study are also in accordance with these earlier findings. The higher K content were observed in accessions/clones already confirmed as drought tolerant based on earlier physiological, biochemical and preliminary germplasam screening in the drought stress area, Dapchari. Therefore the drought tolerance of the accessions/clones was proven and we have observed the higher K content in the rubber leaves in known drought tolerant accessions/ clones and enhanced leaf K during stress and pre-stress which could be an indication of the adaptations to drought tolerance in *Hevea* also. Further detailed studies could provide more results to confirm the observations.

CONCLUSION

High concentration of K in the leaves in all the seasons were recorded by MT 43, RO 2153, MT 4788, RRII 430, RRII 414. The drought tolerant accessions/clones MT 1649, MT 5100, MT 4788, MT 1681, MT 43, RO 2153, 93/92, 93/225 and RRII 430 which recorded high K during pre-stress and stress is an indication of adaptation to drought stress. Five accessions/clones *viz.* MT 4788, MT 43, RO 2153, RRII 430, RRII 414 were classified as most potential drought tolerant clones with higher K content by scatter diagram. Significant difference between seasons and enhanced K content in clones during stress is an indication of adaptation to drought stress in *Hevea*. The observed high K in already identified drought tolerant germplasm accessions/clones and drought tolerant check clone supports the high leaf K is related to drought tolerance in rubber clones. Identification of drought tolerant clones by K accumulation can be used as one of the screening tool for drought tolerance.

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