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Drought resilient *Flemingia semialata* Roxb. for improving lac productivity in drought prone ecologies

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Abstract

A drought tolerant line of *Flemingia semialata* (FS-S) has been identified from natural population as a better host plant for *kusmi* lac production and summer sustainability. FS-S had significantly higher fresh biomass, dry matter, broodlac and scraplac than local line (FS-L). Photosynthetic rate and stomatal conductance were also higher in FS-S line and it was fertilizer responsive too. It is more efficient in starch degradation than the local line which ultimately supports lac insect development. The high level of total sugar maintained by FS-S line is due to high chlorophyll content including carotenoid. Ascorbate peroxide and catalase (biochemical indicators for draught tolerance) was also higher in selected line.

Key words: Drought resilience, *Flemingia semialata*, lac productivity

Introduction

Lac consists of a resin, a pigment, a wax, traces of proteinaceous materials, inorganic salts and a few odoriferous substances. The water soluble lac dye-used as a cosmetic in human decoration, for dyeing wool and silk and to stain leather (Panda 2000).· Lac wax is used in coating fresh fruits of apple and orange to increase shelf-life. Aleuritic acid, the principal material in the resin, is extensively used as a starter in perfumery industry (Alleyne and Hagenmaier 2000). In recent years, lac is used in coating pharmaceutical products for absorption in the hind gut and not in the stomach of humans. Lacquer, a product derived from lac, is useful in realizing an elegant sheen to painted wooden products.

Lac, commercially important resin, is secreted by tiny gregarious lac insect, *Kerria lacca* Kerr, thriving on various host plant. Two strains of lac insect i.e., *kusmi* and *rangeeni* are mainly grown on three tree host species, *kusum, ber* and *palas*. Because of slow growing nature corroborating time consuming of these three tree hosts, discourage the lac grower from raising of new plantation. Keeping in view of the slow growth habit of the traditional host species; a potential fast growing bushy plant species *Flemingia semialata* Roxb. belongs to family papilionaceae, has been identified for intensive lac cultivation during winter season as *kusmi* lac. *Flemingia semialata*, a bushy plant has been identified as productive host for *kusmi* strain of Indian lac insect, *Kerria lacca* Kerr, but limitation of water and high temperature (abiotic stress) and stress rendered by lac insect on host (biotic stress) affects lac insect survival and lac yield in summer. This host will cater the increasing global demand of lac through increased lac production. It is a perennial, erect, fast growing and input responsive lac host, grown up to height of 3 meters depending upon plant management. Being a leguminous plant species, *F. semialata* capacitate to fix atmospheric nitrogen in the soil, thereby enriching soil fertility. Further, farmers are facing problem in lac cultivation during summer season due to severe mortality of lac insect due to biotic as well as abiotic stress imposed by lac insect on this host plants. Lac cultivation on *F. semialata* is possible in summer under assured irrigation facility which is meager. It was observed that the lac growers are facing problem of availability of *kusmi* broodlac (initial seed material) for winter crop (*aghani* crop from July-February), due to limited lac cultivation on *kusum* host.

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Plants have some inherent adaptations for its survival in the diverse environment (Díaz Sandra et al. 1998; Reich et al. 2003). Plant experiences several abiotic stresses *viz.,* high and low temperature, drought, water logging, salt, excessive irradiation and biotic stresses *viz.,* pathogens, nutrient competition, and grazing (Tucker 2010). The generation of novel plant varieties displaying tolerance to abiotic stress is highly expected to cope with the unfavourable environmental condition (Beatriz et al. 2011). In the recent past, intermittent drought, erratic and irregular rainfall became a common phenomenon in the semiarid and arid tropic might be due to climate change. Drought resilient plants will be another option to mitigate this problem. But breeding for increased adaptation to drought is, however, a challenging task due to various complexities associated to drought adaptation mechanism, uncertainty in timing, intensity and duration of stress; and a large genotype x environment interaction. Henceforth, keeping view of the severity of this situation, it is needed to harness drought resistant/ tolerant line of *F. semialata* for better harvest of *kusmi* lac during summer. The present study was conducted to investigate physiological and morphological/biochemical characteristics to produce higher yield of lac from a favourable and suitable tree, specially *F. semialata*.

Materials and methods

Population of *F. semialata* had been procured from ICRISAT, Hyderabad and planted at institute research farm, ICAR-Indian Institute of Natural Resins and Gums, Ranchi. The plant is often cross pollinated hence a natural variation exists in plants. To harness the natural variation in this population, 50 morphologically promising plants were selected in 2006 under rainfed management condition and selfed. Altogether 250 seedlings from selfed seeds of selected plants were raised in July 2007 in rainfed situation. Summer *kusmi* strain was inoculated on these plants with different insect settlement load in February 2008. Only 5 plants survived at 50% lac insect load. Seeds were collected from surviving plants and raised 90 plants in summer 2008. Only 81 selected plants survived after lac insect inoculation (biotic stress) in rainfed condition. In summer 2009, again *kusmi* lac crop raised on 81 plant progenies but due to intense high temperature, insufficient rainfall and severe leaf fall all lines showed a poor performance for *kusmi* lac. Only 70 plants survived with lac in this situation. In summer 2010 and 2011, the performance of *kusmi* lac summer sustainability was assessed on 70 plant progenies in rainfed situation. Keeping in view the optimum moisture condition of host plant at critical stage of sex differentiation of lac insect, selected plants were again assessed for *kusmi* lac production and sustainability of lac insect under limited irrigation condition in two consecutive seasons summer and winter during 2013 and 2014. In our experiments local line was used as check.

Data on each bush were recorded for fresh and dry biomass, shoots number/bush, leaf number/bush, mortality of lac insect, broodlac and scrapedlac. Photosynthetic rate and stomatal conductance of leaves was measured in selected and local lines of *F. semialata.* These were computed with the help of CI-340 hand-held Photosynthesis System, CID, Inc, USA. A study on biochemical parameters *viz*., protein, starch, free phenol, sugar and chlorophyll content in leaves was carried out in selected *F. semialata* line *vis-à-vis* local *F. semialata*. Both the lines were evaluated for winter *kusmi* crop during 2013-14 and 2014-15. Broodlac was inoculated in July and the data recorded for six months (August-January) were pooled over inoculation vs control and year and used for factorial analysis. Starch content was determined as described by McCready et al. (1950) utilizing D-glucose as standard and expressed as mg g-1 DW. Total sugar was determined by Nelson's arsenomolibdate method (Nelson 1944) using improved copper reagent of Somogyi (1952). Soluble protein was estimated by Lowrey's method (Lowrey et al. 1951). Total (free) phenol was determined using Folin Ciocalteau reagent method as described by Brey and Thorpe (1954). Chlorophyll content was estimated by DMSO method as suggested by Hiscox and Isrelstam (1979) using formula given by Lichtenthaler and Wellburn (1983). Ascorbate peroxidase and Catalase reduces hydrogen peroxide (H_2O_2) to water (H_2O). Therefore, plays a crucial role as a signalling molecule in various physiological processes. Keeping this in mind an antioxidant (Ascorbate peroxidase and Catalase activity) study was conducted during May 2013 (summer) in selected *F. semialata* line for drought tolerance vis-à-vis local *F. semialata* in inoculated as well as un-inoculated condition to show the superiority of selected line in terms of tolerance to stress over local line. The ascorbate peroxidase and catalases responses were analyses by Nakano and Asada (1981) and Aebi (1984), respectively in May under severe drought situation. Standard statistical packages were used to draw conclusive result.

Results and discussion

Many morphological, physiological and biochemical traits have been altered by the intense selection pressures for higher yields. These changes indicate that selection for high-yielding genotypes has generated indirect selection pressures on the altered traits. The study of high-yielding lines might reveal specific traits and genes associated with the higher yield. With this information, breeders could explicitly select for yield-enhancing traits to improve yields further.

Morphological changes in selection

The *F. semialata* plants pruned in July and grown in rainfed condition were ready for summer *kusmi* lac cultivation in February. Subsequently plants pruned in February and grown in summer were used for winter *kusmi* lac cultivation in July. Selected line of *F. semialata* (FS-S) had significant variation in morphological traits (Table 1). In rainfed condition, plant height ranged from 129 (local) to 147 cm (selected). Leaves are trifoliate and leaflet elliptic acuminate and it varied from 70 (local) to 93(selected). Number of tillers ranged from 5(local) to 7(selected) but it may go beyond that depending upon crop management. Seeds were round, black with 100 seed weight of 2.7 g (local) to 2.9 g (selected).

Fresh weight of selected line of *F semialata* (FS-S) was observed significantly higher than local *F semialata* (FS-L) during crop season viz., summer and winter over the years. Season affect significantly on fresh weight of plants and it was higher in winter than summer irrespective of selection.

Table 1. Morphology of *F semialata* over season and year

Semialata	Plant height (cm)	No. of tillers	Leaves/ bush	100-seed weight (g)
FS-S	146.5	6.9	93.0	2.9
FS-L	129.2	5.1	70.0	2.7
% increase	13.4	35.3	32.9	7.4
CD at 5%	14.2*	1.2*	16.3*	0.4^{NS}

FS-*F. semialata*, S-selected, L-local

FS-S had fresh weight of 809 g (in summer) and 1316 g/ bush (in winter) and it was 21% and 38% higher than FS-L, respectively (Fig. 1). FS-S had also significantly higher dry weight as compared to FS-L over year. Summer crop gained more dry weight than winter crop. Dry weight of FS-S was recorded as 504 g and 304 g/bush during summer and winter season, respectively and it was 24% and 19% higher than FS-L. Drought affected significantly dry mass accumulation (Yanbao et al. 2006). Zokaee et al. (2014) observed that drought stress caused a significant reduction in plant growth parameters such as fresh and dry weights of plant organs, leaf number and total leaf area in all almond species. It has suggested that yield performance over a range of environments should

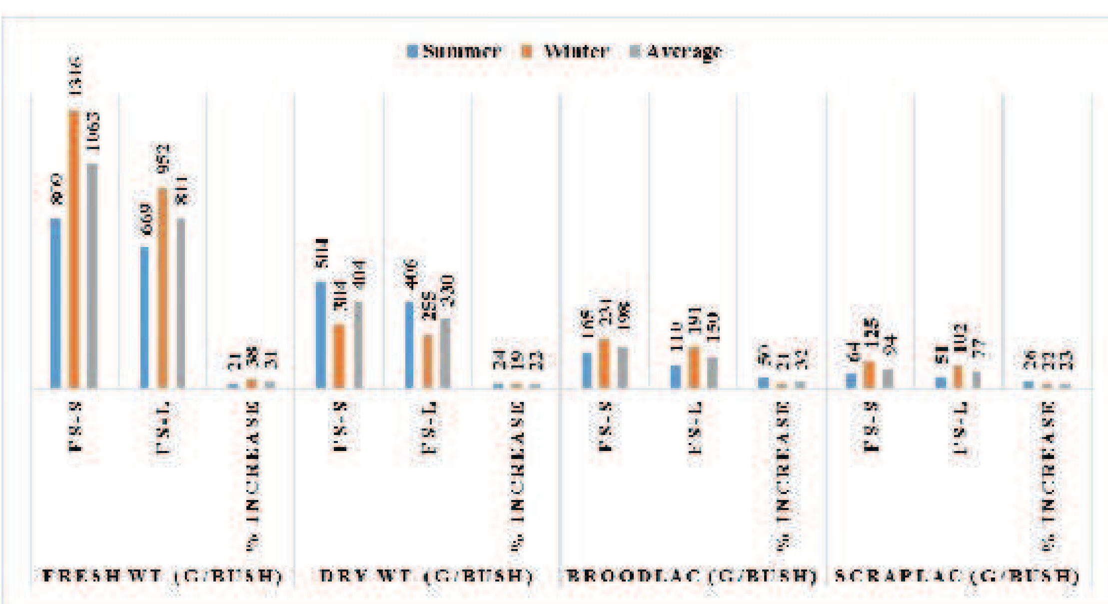

Fig. 1. Green and dry weight and lac production potential in F. *semialata*

be used as the main indicator for drought tolerance (Voltas et al. 2005). In lac cultivation, higher lac yield is indicator of drought tolerance in this bushy host plant.

Broodlac yield (BL) was recorded higher in selection line (FS-S) over year with average gain of 50% over FS-L in summer crop (Fig. 1). However, the differences in BL production was narrowed (21%) in winter due to effect of single factor i.e., stress rendered by feeding lac insect on host. BL was also significantly higher in winter than in summer irrespective of selection and higher in FS-S irrespective of season. Scrapedlac (SL) is raw material for lac industry and it was higher in selection (FS-S) than local. The differences among selected and local *F. semialata* for SL yield was higher in summer and selection gained 26% in summer as compared to 22% gain in winter.

Physiological changes in selection

After the use of recommended dose of fertilizer (RDF) in the experimental plot of *F. semialata*, higher photosynthetic rate was observed in the selected (8.1 µmole/m^2/s) than the local (4.7 µmole/m^2/s) *F. semialata* plant. Likewise, stomatal conductance was also observed higher in the selected (125.2 m mole/m^2/s) than the local (69.6 m mole/m^2/s). This is pertinent to note that the trend of recorded data of photosynthesis rate and stomatal conductance was the same as seen under using recommended dose of fertilizer except in case of stomatal conductance where local FS showed higher value (109 m mole/m^2/s) than that of selected FS (74.2 m mole/m^2/s).

The value of stomatal conductance of leaves of the plant was correlated across diverse environments. This is because photosynthetic rate is a function of intercellular CO_2 concentration (c_i), which in turn a function of stomatal conductance (Radin et al. 1988). Although these two traits were non-significant at recommended fertilizer application and at no fertilizer level but the interaction between fertilizer response and photosynthesis rate and stomatal conductance was found to be significant. It indicates that selected line of *F. semialata* is more responsive to fertilizer application. Increase in net photosynthesis is closely correlated to increase in stomatal conductance (Jarvis and Davies 1998). Applying chemical fertilizer and organic fertilizer increased the leaf photosynthetic rate and stomatal conductance (Wang et al. 2012). Water deficit significantly affected gas exchange and chlorophyll fluorescence parameters. It reduced the net photosynthesis rate, transpiration rate and stomatal conductance (Bogale et al. 2011). Fischer et al. (1998) studied changes in stomatal conductance associated with selection progress. They found that stomatal conductance and maximal rates of photosynthesis were positively correlated with increased yields of advanced cultivars.

Biochemical changes in selection

Biochemical parameters *viz*., total sugar, soluble protein, starch and chlorophyll content in leaves of *Flemingia semialata* plants were significantly different in both selected and local lines. Selected *F. semialata* has also significantly higher status of these biochemical parameters in leaves irrespective of inoculation (Table 2). Total sugar level showed increasing trend from August to November during the life cycle of lac insect in both the lines. It reduced drastically in January in both the lines. The FS-L felt more stress during crop maturity period of lac insect during December- January. Selected line (FS-S) had 14.9% higher total sugar than local line. FS-S maintains high level of starch throughout the crop season. The recorded data revealed a low level of starch in local line (22.4 mg/g fw) than the selected line (25.5 mg/g fw) and the increase in selected line was about 14 % over FS-L (Fig. 2). Selected lines of *F. semialata* had higher protein content (70.1 mg/g fr.wt.) than local (68.9 mg/g fr.wt).The increase in protein was about 1.7 % in selected line over local line. Total free phenol level showed increasing trend throughout the life cycle of lac insect in both the lines. The FS-L felt more stress during initial growth period of lac insect during September to November, however FS-S showed more stress during lac crop maturity in December and January. Selected line had 3.74% higher free phenol

Table 2. Starch, protein, phenol and total sugar in selected *F. semialata*

Month	Starch		Protein		Phenol		Total sugar	
	FS-S	FS-L	FS-S	FS-L	FS-S	FS-L	FS-S	FS-L
August	9.05	8.81	43.65	43.18	6.61	6.60	5.93	2.74
September	34.94	27.12	57.85	57.22	8.37	8.94	9.15	12.23
October	23.00	19.11	65.44	64.63	7.81	8.26	13.81	9.53
November	37.69	35.30	77.61	76.88	9.99	10.46	24.29	22.47
December	25.49	24.17	79.49	85.78	11.51	10.05	19.74	16.76
January	22.63	19.59	96.43	85.94	17.24	16.19	3.53	2.75
CD (5%)	1.06*		1.48*		0.20*		1.65*	

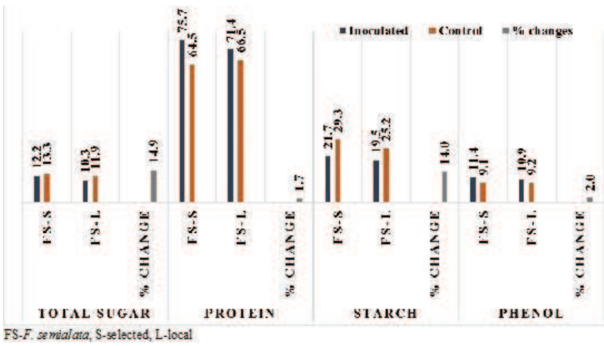

Fig. 2. Extent of variation in biochemical parameters in *F. semialata* lines

than local line. All these biochemical parameters differed significantly in lines of *Flemingia semialata*, over month and over year.

Selected FS had higher chlorophyll A, chlorophyll B, total chlorophyll and carotenoids in comparison to local FS (Table 3). The difference in chlorophyll

Table 3. Chlorophyll component in leaves of *F. semialata* lines

Months	Chl. ll A		Chl. ll B		Total chl.		Carotenoid	
	FS-S	FS-L	FS-S	FS-L	FS-S	FS-L	FS-S	FS-L
August	4.25	3.97	3.35	2.12	7.60	6.10	6.68	6.50
September	4.18	4.02	4.05	3.34	8.22	7.36	6.80	6.50
October	2.80	2.25	0.86	0.72	3.66	2.97	3.96	3.35
November	4.05	3.85	2.88	1.79	6.93	5.63	7.30	6.74
December	2.36	2.01	2.12	1.65	4.47	3.66	5.35	4.75
January	2.74	2.73	2.26	1.33	4.00	4.06	6.89	6.14
Mean	3.40	3.14	2.42	1.83	5.81	4.96	6.16	5.68

Chl. = Chrophyll

component was higher during initial growth of lac insect but tending to decrease in subsequence growth and it was non-significant during maturity of lac insect in Dec-Jan. Greenish stem is an important trait in several crops (e.g. maize, rice, sorghum) which has been extensively used in plant breeding to improve yield potential and yield stability in all environments, including drought-prone areas (Campos 2004, Tollenar and Wu 1999). Drought affected the rate of photosynthesis, accumulation of total soluble sugars, chlorophyll "a" and "b" and also carotenoids. It also affected the growth and the photosynthetic pigments which ultimately reduced the photosynthetic rate and physiology and biochemical processes (Usman 2013).

Oxidative stress indicators

The present study showed more ascorbate peroxidase activity in selected *F. semialata* (FS-S) line for drought tolerance when inoculated with lac insect than local line. In un-inoculated condition the same trend was observed. This shows that the selected line has more ability to withstand any stress over local line (Table 4). However, the reverse trend was observed in

Table 4. Oxidative stress parameters in *F. semialata* lines

F. semialata lines	Ascorbate peroxidase (mM/min/ g fr. wt.)	Catalase (mM of H2O2/min/g fr. wt.)
FS-S-I	80.71	0.72
FS-S-C	52.74	0.59
FS-L-I	45.71	1.04
FS-I-C	41.19	0.14

Catalase activity wherein Catalase activity was more in local line. Since Catalase activity is considered less efficient than ascorbate peroxidase in scavenging H_2O_2 our results strengthen that selected line has efficiency to sustain lac production in summer condition.

The early plant responses to attacks by phytophagous triggers events such as protein phosphorylation, membrane depolarization, calcium influx and release of reactive oxygen species (ROS, such as hydrogen peroxide (H_2O_2)) (Garcia-Brugger 2006). This is associated with localized cell death and necrotic lesions on tissues were they feed (Philippe et al. 2010). The cabbage aphid (*Brevicoryne brassicae*) showed up-regulation of several genes encoding proteins involved in ROS detoxification (e.g. ascorbate reductases and L-ascorbate oxidase, copper protein precursor, glutathione S-transferases and glutathione S conjugate transporters, peroxidase precursors) in *Arabidopsis thaliana* when infested.

It may be concluded that selected *Flemingia semialata* (FS-S) had significantly higher fresh biomass, dry matter, broodlac and scrapedlac than local line (FS-L). Photosynthetic rate and stomatal conductance were also higher in selected line and more responsive towards fertilizer application. FS-S line maintained high protein, phenol, total sugar, starch and chlorophyll component than the local line. Ascorbate peroxide (biochemical indicators for draught tolerance) was also higher in selected line. Hence, FS-S identified as drought tolerant line used for *kusmi* lac production and summer sustainability, will facilitate lac growing farmers to get good *kusmi* lac in summer season.

Authors' contribution

Conceptualization of research (JG, VDL, SG); Designing of the experiments (JG, VDL, SG); Contribution of experimental materials (JG, SG); Execution of field/lab experiments and data collection (JG, VDL, SG, VS, NKS); Analysis of data and interpretation (JG, VDL, SG); Preparation of manuscript (JG, VDL, VS, NKS).

Declaration

The authors declare no conflict of interest.

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