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Physio-biochemical markers for selecting water logging tolerant mulberry genotypes

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ABSTRACT

Water logging not only restricts soil aeration but also limits growth of the root system, stimulates toxic metabolism, disturbs hormone metabolism and prevents the normal functioning resulting in reduction in leaf yield and quality of mulberry. Under prolonged water stagnation heavy mortality occurs. To overcome this problem, one important way is to develop mulberry genotypes which have higher potential to tolerate flood /water logging condition. Accordingly, to chalk out a hybridization programme, response of a total of 120 genotypes was studied after simulating the plants under water-logged condition for 15 days. Various relevant physio-biochemical traits were measured, viz. net photosynthetic rate (NPR), nitrate reductase activity (NRA), chlorophyll content, soluble protein content, abscisic acid (ABA) and membrane stability. Attempt was made to identify important economic characters and physiological traits susceptible to high moisture stress. Three physiological traits: NRA, membrane stability and ABA content, were identified as the most important traits to select the flood / water logged condition tolerant mulberry genotypes. Correlation studies revealed significant positive correlations between economically important parameter- leaf yield in stress condition, with three physiological parameters: NRA, abscisic acid content and membrane stability expressing highest r value of 0.72 with ABA. On the basis of overall findings, a total of 15 parents, comprising 4 male and 11 female genotypes, were selected as promising parents for taking up breeding programme to develop F_1 progenies.

Key words: Flood tolerant variety, mulberry, water logging

Availability of moisture in the soil is important for growth and development of plants. Most of the tropical lowlands are humid and marshy due to high rainfall and poor drainage system, plants growing there face the problems related to excessive moisture in the soil. Stagnation of water in the low lying areas not only restricts soil aeration but also (a) impairs the root respiration by slowing down nutrient and water uptake, (b) limits growth of the root system, (c) stimulates toxic metabolism, (d) disturbs hormone metabolism, and (e) prevents the normal functioning of essential biological, chemical and physical processes associated with fertile and productive soils. The renewal of soil air is not only important from the point of maintenance of an optimum level of O_2 and CO_2 but also for exclusion of byproducts of anaerobic decomposition, such as hydrogen sulphide, methane, hydrogen, etc., and the stresses produced by ethylene which accumulates in the soil. In addition, high temperature further aggravated the problem as more O_2 is required to produce a given growth response than at lower temperature. This is partly due to the lower solubility of O_2 in water and partly due to the increased rate of O_2 consumption by roots and soil micro-organisms. Micro-organisms affect the distribution of O_2, influence the supply of nutrients (e.g. nitrate) and under anaerobic condition produce compounds which are extremely toxic to plants (such as sulphide and butyric acid).

Alcohol dehydrogenase was taken as a selection criterion as in absence of oxygen it helps by its oxidizing power. Rapid increase in alcohol dehydrogenase is considered as a powerful adaptive mechanism for water logging tolerance (Cao and Cai, 1991). Nelson *et al.* (1983) used triphenyl tetrazoliumchloride-reduction method which measures flooding tolerance by determining the rate of root respiration during stress, for selecting *Phaseolus* bean genotypes.

Sethiya *et al.* (1986) used nitrate reductase as a selection criterion. They evaluated tolerant, susceptible and fairly tolerant cultivars of sugarcane for nitrate reductase content in the top unfurled leaves. In the tolerant cultivars CoH12, Co7717 and CoH14, nitrate redutase activity declined to zero in 20-30 days after the initiation of water logging whereas in susceptible cultivars, CoH48 and Co7314, such decline took only 10 days. On return to normal condition, the nitrate reductase activity resumed in tolerant cultivars but not in susceptible cultivars.

Auxin production or transport or both are affected by flooding. Flooding the roots of sunflower plants results in increased auxin content in the shoot (Phillips, 1964). An anaerobic environment inhibits auxin transport in coleoptiles of maize and oats (Goldsmith, 1968; Wilkins and Whyte, 1968) which might explain the build-up of auxin in shoots observed by Phillips (1964). On the other hand, IAA-oxidase activity may be restricted just by lack of O_2 or due to the accumulation of a phenolic inhibitor of IAA-oxidase (Kefford, 1962). In addition, ethylene originating from the root tissue or from activities of soil micro-organisms may interfere with various auxin controlled processes.

Increase in concentration of ethylene in the tissues of various species has been recorded. Perhaps other symptoms of flooding such as premature leaf senescence and chlorosis, and adventitious root

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production could be affected by anoxia-induced ethylene production (Kawase, 1976; El Beltagy and Hall, 1974; Jackson and Campbell, 1976). Ethylene concentration in sunflower cuttings increased 5-fold within 6 h after submergence in distilled water and then declined (Kawase, 1976).

Flooded bean seedlings contain more ABA than the nonflooded ones (Wright and Hiron, 1970). Wright and Hiron (1972) suggested that this increase probably results from an incipient wilting of leaves that results due to waterlogging. Increased levels of ABA influence GA-biosynthesis, stem elongation and senescence and abscission. Significant increases in ABA content of pea root and shoot tissues have also been observed after 22 and 36 hours of flooding, respectively (Zhang and Davis, 1987). In three days, ABA content of pea shoots increased up by 10-fold of the endogenous level (Jackson and Hall, 1987).

Mulberry grows well up to 45-50% of available soil moisture but suffers from excess moisture. The major silk producing districts of West Bengal, *viz.* Malda, Murshidabad, Birbhum and adjacent areas of Bihar are often inundated by flood waters from the river Ganges and its tributaries causing considerable loss in leaf yield and thereby silk production. Under prolonged water stagnation heavy mortality of plants occurs. To overcome this problem, development of high moisture stress-tolerant mulberry genotypes becomes essential. Accordingly, response of a total of 120 genotypes belonging to germplasm, elite and pipeline mulberry genotypes towards high moisture stress was studied for relevant economic (leaf yield and leaf fall %), and physio-biochemical traits [net photosynthetic rate (NPR), nitrate reductase activity (NRA), chlorophyll content, soluble protein content, abscisic acid (ABA) and membrane stability] to identify / select prospective parents and also to find out important economic characters and physiological traits influenced by high moisture stress. Therefore, a pre-breeding strategy was adopted to identify suitable parents of mulberry having excessive moisture stress tolerant characteristics.

MATERIALS AND METHODS

In the present study a total of 15 genotypes (11 GPB and 4 elite lines) were selected out of a total of 120 germplasm accessions, elite lines, pipe lines and tetraploids, maintained at Central Sericultural Research and Training Institute, Berhampore, West Bengal, India on the basis of leaf yield, number of roots, root length and its anatomical characters for further physiological evaluation related to excessive moisture stress tolerant characteristics. The experimental site was at Berhampore in Eastern India at 24° 6' N latitude and 88° 15' E longitude at an altitude of 19.0 m. above msl. The average maximum and minimum temperatures are 32.2° C and 20.6° C, average maximum and minimum relative humidity are 90.69 and 62.01 per cent, respectively, with an average annual rainfall of 1377 mm, mainly distributed from May to November. The soil is of Gangetic alluvium type with pH 6.9; EC 0.12 dS/m and OC 0.56 g kg^{-1}.

All important parameters related to cold tolerant characteristics *viz.*, nitrate reductase activity (Hageman and Hucklesby, 1971) abscisic acid content (Wright and Hiron, 1970), membrane stability (Tyagi *et al.* 1990), net photosynthetic rate during winter (LI-COR model 6200; Licor Instrument Inc, USA), total chlorophyll level (Arnon, 1949) and total soluble protein content (Lowry *et al.* 1951) of 15 selected genotypes were measured.

Data analysis was done and range of values, mean, standard deviation, CD and CV% were calculated. In addition to these, correlation studies were made for leaf yield with all the physio-biochemical parameters like membrane stability, NRA and abscisic acid content. Finally on the basis of ABA values of genotypes as this showed highest correlation with leaf yield, the genotypes were grouped into three categories as high, medium and low (Arunachalam and Bandopadhyay, 1984) for taking up breeding programme in maximum combination.

RESULTS AND DISCUSSION

Values of mean leaf yield, leaf fall%, number of root per plant, length of longest root, root anatomical characters like thickness of cortex, medullary ray and cork, net photosynthetic rate (μmol $m^{-2}s^{-1}$), nitrate reductase activity (μ mol NO_2^- h^{-1} g^{-1} fr. wt), total chlorophyll content, total soluble protein, abscisic acid content and membrane stability of 15 genotypes presented in Table 1 and 2. Wide variations among the genotypes with respect to each of the parameter under study were observed, thereby justifying the inclusion of these parameters in breeding programme involving these genotypes.

The maximum leaf yield / plant (average of 5 crops) 238.33g and minimum leaf yield / plant (155 g) was found in Kajli. Days to sprout after pruning was found minimum in Kanva-2 (5.78 days) and maximum in V-1 (21 days); leaf fall% was recorded minimum of 15.00% in C-2028 and maximum in *M.laevigata* (33.33%). Number of roots per plant and length of longest root were recorded maximum in C-2028 (28.00 and 64.33 cm respectively) and minimum in Kajli (10.67 and 17.67 cm respectively).

Sethiya *et al.* (1986) used nitrate reductase as a selection criterion. They evaluated tolerant, susceptible and fairly tolerant cultivars of sugarcane for nitrate reductase content. In the tolerant cultivars nitrate reductase activity declined to zero in 20-30 days after the initiations of water-logging whereas in susceptible cultivars this decline took only 10 days. On return to normal condition, the nitrate reductase activity resumed in tolerant cultivars but not in susceptible cultivars. Similar observation has been found in C-2028.

Among the genotypes screened, Abscisic acid (56.90 nmol g^{-1} fresh wt) was found higher values in C-2028. Information on the physiological aspects of plants due to flood condition revealed that production or transport or both of the hormone like auxin are affected by flooding. The anaerobic environment due to flooding inhibits auxin transport in plants. On the other hand, IAA-*oxidase* activity also restricted due to lack of O_2 or accumulation of a phenolic inhibitor of IAA-*oxidase* in the plants. Besides, ethylene originating from the root tissue or from activities of soil micro-organisms may interfere with various auxin controlled processes, found minimum in C-2028. Thus, the water logging-resistant mulberry variety should have a mechanism to restrict the rise in ethylene concentration which was found in agreement with the findings of Dodds *et al* (1984) in *Vicia fava* where highest resistance water logging variety exhibited rapid conversion of ethylene to ethylene oxide.

A direct correlation between excessive moisture stress tolerance and ABA has been reported in bean seedlings (Wright and Hiron, 1970) which is in accordance with our observation in mulberry where a significant positive correlation existed between ABA content and leaf yield (r=0.72**) (Table 3).).

According to Arunachalam and Bandyopadhyay (1984), all the15 selected genotypes were grouped in 3 categories on the basis of values of abscisic acid content. The first group was designated as high and comprised two genotypes with ABA content > (mean + SD) to (mean + 2 SD). The medium group having 4 genotypes with ABA values between mean and (mean + SD) and the low group comprised 9 genotypes with ABA content less than mean. Group wise genotypes were further identified according to their sex. The details of genotypes identified and grouped as parents are given in Table 4.

Therefore, as a pre-breeding strategy, the genotypes having excessive moisture stress-tolerant characteristics were identified and grouped in different classes so that suitable crossings could be made in different combinations like high × high, high × medium and high × low to get desired F_1 progenies.

Table 1: Mean of net photosynthetic rate, nitrate reductase activity, soluble protein content, total chlorophyll, abscisic acid content and membrane stability % along with relevant statistical parameters of 15 mulberry genotypes

Mulberry genotypes	NPR	NRA	Soluble protein content	Total chlorophyll	ABA	Membrane stability
C-2028	13.58	7.86	28.63	2.22	56.27	86.33
S-1532	12.62	7.39	23.27	1.83	47.13	83.00
Shrim-2	14.23	10.35	28.07	2.07	34.00	84.67
Phillipines	11.67	9.51	28.97	1.81	24.03	78.33
C-2036	10.50	9.73	28.57	2.12	22.30	76.00
M.indica HP	9.23	9.53	28.73	1.97	20.90	77.67
China White	8.03	7.30	26.07	1.77	28.30	71.67
Berhampore-A	11.67	8.84	28.03	1.88	28.00	80.67
Rotundiloba	9.13	6.56	22.67	1.66	16.77	75.00
Punjab Local	10.19	7.43	24.80	1.88	25.57	73.00
M.laevigata	12.03	8.82	28.73	1.92	38.23	75.33
Kajli (OP)	14.10	9.80	27.67	2.16	41.57	84.67
Bishnupur-4	9.23	6.96	24.83	1.69	21.50	73.33
Bogura-4	7.57	5.64	22.23	1.61	18.77	77.67
Kajli	9.30	8.42	22.60	1.71	37.57	82.67
Mean	**10.87**	**8.28**	**26.26**	**1.89**	**30.73**	**78.67**
CV%	**3.40**	**4.17**	**2.84**	**2.61**	**3.02**	**1.24**
LSD(0.05)	**0.62**	**0.58**	**1.25**	**0.08**	**1.55**	**1.64**

(Units - NPR: $\mu mol\ m^{-2}s^{-1}$, NRA: $\mu\ mol\ NO_2^-\ h^{-1}\ g^{-1}$ fr. Wt, Sol. pro. and chl. content : mg g^{-1} fr. Wt., ABA : n mol g-1 fr. Wt.)

Table 2: Correlation coefficient of leaf yield with Abscisic acid, nitrate reductase activity and membrane stability

Parameters	Leaf yield
Abscisic acid (n mol g-1 fr. Wt.)	0.72**
Nitrate reductase activity (μ mol NO_2^- h^{-1} g^{-1} fr. Wt)	0.51**
Membrane stability (%)	0.54**

*** Significant at 1% level of significance*

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