Evolution of Low Temperature Stress Tolerant Mulberry genotype for Eastern and North-Eastern plains of India

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Abstract: Eastern and north-eastern part of India, which is sericulturally important zone, suffers from low temperature conditions from November to January with temperature below 15°C. Out of 5 commercial silkworm rearings in a year in this zone, two seasons fall during this period. This period is favourable for silkworm rearing but is unfavourable for mulberry growth resulting into quantitatively and qualitatively poor leaf yield due to prevailing low temperature and humidity. To overcome this problem, a programme was taken up to develop low temperature stress tolerant mulberry genotype(s) through conventional method of breeding. To identify suitable parents study of a total of 120 Germplasm, elite and pipeline mulberry genotypes was made for relevant economic (leaf yield, leaf fall% and days to sprout), anatomical (number of chloroplast per guard cell) and physiological traits [net photosynthetic rate (NPR), nitrate reductase activity (NRA) and electrical conductivity (EC)]. Character association was determined through correlation / regression analysis between important economical characters and physiological, anatomical and biochemical traits. Correlation between winter leaf yield and days to sprout with three physiological parameters (NPR, NRA and EC) and number of chloroplast/ guard cell, it was found that a positive correlation existed between winter leaf yield and the aforesaid four parameters whereas a negative correlation existed with days to sprout. From sericultural point of view it has been observed that leaf yield suffers mainly due to delayed sprouting or no sprouting during winter season. In this investigation it was found that EC has the highest negative correlation with days to sprout *i.e.*, higher the EC value lesser the days to sprout. On the basis of above investigation EC has been identified as the most important physiological parameter to select the prospective parents to develop low temperature stress tolerant mulberry genotypes. As such, a total of 36 parents were selected and grouped into three categories on the basis of EC values viz., high (6 parents), medium (13 parents) and low (17 parents) for further breeding programme.
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Key words: Mulberry, low temperature stress tolerant, EC, NPR, NRA

1.Introduction:

The importance of low temperature (cold and frost) as one of the most devastating natural calamities capable of killing the lush green luxuriant crops in a matter of hours, was realized long ago and scientists have studied the problem closely. Crop physiologists, ecologists, biochemists, geneticists and breeders have all contributed and the subject has been reviewed several times (Bulavka, 1989; Zhong, 1991).

Mulberry is a perennial tree and thrives well between 20ºC and 30ºC temperature. When the temperature falls below 13ºC the plant stops its growth and enters into a period of dormancy (Anonymous, 1995).

In the Gangetic alluvial soils of West Bengal there are five commercial cocoon crop seasons in a year under irrigated condition viz., April, July, September, November and February. Out of these five seasons, November and February are favourable and remaining three crops are unfavourable from silkworm rearing point of view. It has been found that the total leaf yield of mulberry during the two important commercial cocoon crop seasons in plains of West Bengal is 20-28% of the total leaf yield. Mulberry leaves with superior quality and higher yield are the essential requirement for quality cocoon production. Although, a number of HYVs are available in the field, but the difficulty with those is delayed sprouting after pruning and poor leaf yield during the two favourable commercial silkworm rearing seasons. This is a long realized perpetual problem of Eastern and North-Eastern India affecting the sericultural productivity.

Reports on mulberry evaluation in the temperate countries like China, Russia and Japan revealed that a number of mulberry varieties were developed by the breeders for temperate conditions which have potentiality to sprout and grow well immediately after cold spell. In Russia, two mulberry varieties – Zimestojkij (Filippovich and Strasonova, 1965) and Ukranin-1 (Pryluckyj, 1969) were developed which are tolerant to cold and sustain almost normal growth during the cooler months.

Inheritance of frost resistance in winter wheat was studied early in this country by Nilsson-Ehle (1912) who observed transgressive segregation of this character. Mc Murphy and Rayburn (1992) noted that guard cell size of all the cold-tolerant maize populations was larger than the cell size of the respective non-selected populations. Chloroplast number per guard cell was also higher in all the cold-tolerant populations than in their parental populations. Several workers have determined electrical conductivity of plant sap and have found a direct correlation between cold/frost resistance and electrical conductivity of wheat varieties (Mel'niskii and Bidyukova, 1980; Musich and Kornelli, 1982; Ding, 1984; Brzostowicz *et al*., 1985).

Heritability of cold tolerance in *Phaseolus* beans is 35% (Dickson, 1971). Kolomiets (1980) also confirmed that most hardy winter wheat hybrids were obtained from crosses of the hardy × hardy or hardy × moderately hardy types. Hybrids between hardy and high yielding varieties, such as Mironovka 808 × Krasnodar 39, were considered useful as donors of resistance in crosses.

Puzakova and Kovshova (1980) and Biryukov *et al*., (1982) opined that most cold resistant wheat variety had the highest photosynthetic rate and the least resistant variety had the lowest rate. It has been established by Johnson *et al*., (1976) that Nitrate Reductase (NR) activity can be considered as predictive test for crop yield. In recent years, NR activity has been increasingly used as an additional parameter for identifying high yielding genotypes in several crop plants (Rao *et al*., 1990; Singh *et al*., 1994; Amaresh and Roy, 1995). According to Ghosh *et al.* (2004) NR activity has positive correlation with leaf yield and many of the qualitative and yield attributing characters in mulberry.

Therefore, in the present investigation, as a pre-breeding strategy, it has been attempted to select parents out of a total of 120 Germplasm, elite and pipeline mulberry genotypes having cold tolerant characteristics based on relevant economic (leaf yield, leaf fall% and days to sprout), anatomical (number of chloroplast per guard cell) and physiological traits (NPR, NRA and EC) and to identify relevant parameters most promisingly influenced by cold, for utilization in future breeding programme to develop cold tolerant / resistant mulberry genotypes.

2.Materials and Methods:

In the present study a total of 36 genotypes (25 GPB, 2 elite lines, 2 pipe lines, 6 tetraploids and 1 HYV) 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 winter leaf yield, short sprouting duration and low leaf fall% for further physiological evaluation related to cold tolerant characteristics. The experimental site was at Berhampore in Eastern India at 24º 6' N latitude and 88º 15' E longitude at an altitude of 19.0 m. above msl. The average maximum and minimum temperatures are 32.2º C and 20.6º 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., number of chloroplasts per guard cell, net photosynthetic rate during winter (LI-COR model 6200; Licor Instrument Inc, USA), nitrate reductase activity (Hageman and Hucklesby,1971) and electrical conductivity of leaf sap (Mel'niskii and Bidyukova, 1980) of 36 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 winter leaf yield and days to sprout after pruning with all the physiological parameters like NPR, NRA, electrical conductivity and number of chloroplasts per guard cell. Finally on the basis of EC values of genotypes as this showed highest correlation with leaf yield and days to sprout after pruning during winter, the genotypes were grouped into three categories as high, medium and low (Arunachalam and Bandopadhyay, 1984) for taking up breeding programme in maximum combination.

3. Results and discussion:

Values of mean leaf yield, days to sprout after pruning, leaf fall%, number of chloroplasts per guard cell, net photosynthetic rate (μmol $m^{-2}s^{-1}$), nitrate reductase activity (μ mol NO_2^- h^{-1} g^{-1} fr. wt) and electrical conductivity (dS/m) of leaf sap of 36 genotypes presented in Table 1. 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) 432g and maximum winter leaf yield (February crop) 398 g were recorded in RG-120 and minimum leaf yield / plant (149 g) and minimum winter leaf yield (125 g) were 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 5.24% in T-2 and maximum in T-21 (15.18%). Number of chloroplasts per guard cell was recorded maximum in T-22 (18.67) and minimum in Kajli (7.33). Net photosynthetic rate was recorded maximum in Shrim-2 (16.98 μ mol $m^{-2}s^{-1}$) and minimum in Kajli (7.94 μ mol $m^{-2}s^{-1}$). Nitrate reductase activity was found maximum in RG-120 (12.50 μ mol NO_2^- h^{-1} g^{-1} fr. wt.) and minimum in Dudhia Red (5.10 μ mol NO_2^- h^{-1} g^{-1} fr. wt.). The electrical conductivity of leaf sap was recorded maximum in Sujanpur-5 (3.53 dS/m and minimum in V-1 (1.80 dS/m).

In the present investigation the correlation between physiological parameters *i.e.*, electrical conductivity, net photosynthetic rate, nitrate reductase activity and number of chloroplasts / guard cell and two important growth and yield parameters *viz.*, winter leaf yield and days to sprout was studied. (Table 2).

According to McMurphy and Rayburn (1992) number of chloroplast / guard cell was higher in all the cold tolerant populations of winter wheat. Similar trend was also found in mulberry where a significant positive correlation existed between number of chloroplasts per guard cell and winter leaf yield (r = 0.68**) whereas a negative correlation was found between days to sprout and chloroplast number / guard cell (r = -0.48**) which is desired for a cold tolerant genotype (Fig 1 and Fig 2).

Puzakova and Kovshova (1980) and Biryukov *et al.* (1982) opined that most cold resistant wheat variety had highest photosynthetic rate and the least resistant variety had the lowest rate which corroborates our results in mulberry where a significant positive correlation existed between net photosynthetic rate and leaf yield (r = 0.82**) and negative correlation (r = -0.53**) with days to sprout (Fig 3 and Fig 4).

It has been established by Johnson *et al.* (1976) that nitrate reductase activity can be considered as predictive test for crop yields. In recent years, NR activity has been increasingly used as an additional parameter for identifying high yielding genotypes in several crop plants (Rao *et al.*, 1990; Singh *et al.*, 1994; Amaresh and Roy, 1995). According to Ghosh *et al.* (2004) NR activity has positive correlation with leaf yield and many of the qualitative and yield attributing characters in mulberry. Similar trend was also found in the present study where a significant positive correlation existed between NRA and leaf yield (r = 0.90**) and a negative correlation (r = -0.53**) with days to sprout (Fig 5 and Fig 6).

A direct correlation between cold tolerance and electrical conductivity has been reported in wheat varieties (Mel'niskii and Bidyukova, 1980; Musich and Kornelli, 1982; Ding, 1984; Brzostowicz *et al.*, 1985) which is in accordance with our observation in mulberry where a significant positive correlation existed between electrical conductivity and leaf yield (r=0.74**) and a negative correlation (r = -0.73**) with days to sprout (Fig 7 and Fig 8).

Therefore, it is revealed that all the physiological parameters are positively correlated with winter leaf yield and negatively correlated with days to sprout after pruning. From Sericultural point of view it has been observed that leaf yields suffer mainly due to delayed sprouting or no sprouting during winter season. In this investigation it was found that EC has the highest negative correlation with days to sprout, *i.e.*, higher the EC values earlier the sprouting. On the basis of above observation EC has been identified as the most important physiological parameters to select the prospective cold tolerant parents.

According to Arunachalam and Bandyopadhyay (1984), all the 36 selected genotypes were grouped in 3 categories on the basis of EC values. The first group was designated as high and comprised six genotypes with EC values > (mean + SD) to (mean + 2 SD). The medium group having 13 genotypes with EC values between mean and (mean + SD) and the low group comprised 17 genotypes with EC values 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 3.

Therefore, as a pre-breeding strategy, the genotypes having cold-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 growth, leaf yield and different physiological parameters along with relevant statistical parameters of 36 mulberry genotypes.
(Units - Leaf yield: g/plant, NPR: µmol $m^{-2}s^{-1}$, NRA: µ mol NO_2^- h^{-1} g^{-1} fr. Wt, EC: dS/m)

Mulberry Genotypes	Days to sprout	Mean Leaf yield	Winter leaf yield	Leaf fall%	C'plast/guard cell	NPR	EC	NRA
M.australis	7.33	377.00	320.00	7.47	11.67	12.98	3.13	9.80
Shrim-2	8.33	351.20	310.00	6.76	15.00	16.98	3.13	9.50
Thailand UL	9.00	217.60	185.00	7.74	9.33	10.21	2.53	7.20
T-22	8.00	334.00	310.00	10.22	18.67	12.62	2.75	10.20
Sujanpur-5	8.33	319.60	290.00	10.37	15.67	16.60	3.53	10.60
Punjab local	10.33	212.00	180.00	6.75	9.00	9.95	2.63	6.50
Sultanpur	10.00	217.40	190.00	5.40	9.67	9.82	2.47	7.50
Kolitha-3	11.67	223.00	196.00	7.60	10.00	9.92	2.53	7.80
M.indica HP	8.00	335.60	290.00	5.27	8.33	11.60	3.03	11.20
KPG-1	7.33	322.00	287.00	7.61	14.33	10.84	3.13	11.60
T-2	8.33	319.60	269.00	5.24	17.33	11.53	2.73	12.20
Mandalaya	8.67	297.80	263.00	7.39	12.00	10.55	2.73	9.90
Kosen	9.67	309.60	271.00	9.05	14.33	12.93	2.83	9.70
CSRS-II	8.00	307.60	265.00	9.39	12.00	12.26	2.77	9.50
Nagaland local	7.33	355.60	272.00	9.61	10.33	11.20	2.73	8.90
Philippines	8.00	321.00	281.00	12.57	11.67	13.25	2.73	8.80
Italian mulberry	10.00	210.20	183.00	14.05	8.67	9.81	2.43	6.80
Kolitha-8	7.33	319.60	254.00	7.90	14.67	13.05	3.03	9.40
Kolitha-9	8.00	315.80	257.00	7.19	14.33	11.61	3.07	10.70
Matigara black	12.33	181.00	150.00	10.41	8.33	8.38	2.30	5.60
T-21	10.33	218.60	176.00	15.18	10.33	8.60	2.63	8.70
T-13	8.33	363.80	310.00	15.07	12.33	13.11	2.83	11.50
T-27	8.33	385.60	324.00	13.57	13.33	14.79	3.07	12.40
Berhampore-B	11.67	194.60	162.00	14.57	8.33	8.03	2.17	5.80
T-7	9.67	226.60	198.00	10.09	10.33	9.73	2.50	7.50
Dudhia red	11.67	173.20	145.00	15.07	7.33	8.22	2.37	5.10
Berhampore-A	11.33	173.40	148.00	8.72	9.00	8.16	2.33	5.60
Kolitha-7	8.33	310.20	267.00	7.50	15.33	14.65	2.93	9.80
Cyprus	11.67	190.60	162.00	13.40	7.33	8.46	2.33	5.40
Kanva-2	5.78	234.40	197.00	9.28	9.00	9.58	2.53	7.30
Kajli	12.33	149.00	125.00	8.01	7.33	7.94	2.20	5.60
Monla	10.33	251.80	210.00	11.08	10.33	9.25	2.63	7.40
Kajli (OP)	10.00	214.60	170.00	6.38	9.00	9.34	2.13	5.80
V-1	21.00	271.00	225.00	6.37	9.00	8.76	1.80	6.40
C-2028	8.67	324.60	256.00	7.84	13.33	13.08	2.93	9.80
C-2038	8.33	432.40	398.00	6.71	11.33	13.10	2.87	12.50
CD at 5%	0.77	15.44	13.25	1.12	1.92	0.69	0.90	0.18
CV%	4.98	4.52	5.24	7.37	10.42	3.85	2.07	1.29
Max. value	21.00	432.40	398.00	15.18	18.67	16.98	3.53	12.50
Min. value	5.78	149.00	125.00	5.24	7.33	7.94	1.80	5.10
Mean	9.55	276.71	236.00	9.36	11.34	11.14	2.68	8.61
SD	2.55	71.90	64.57	3.01	2.97	2.41	0.35	2.22

Table 2. Correlation coefficient of winter leaf yield and days to sprout with electrical conductivity, net photosynthetic rate, nitrate reductase activity and number of chloroplasts / guard cell.

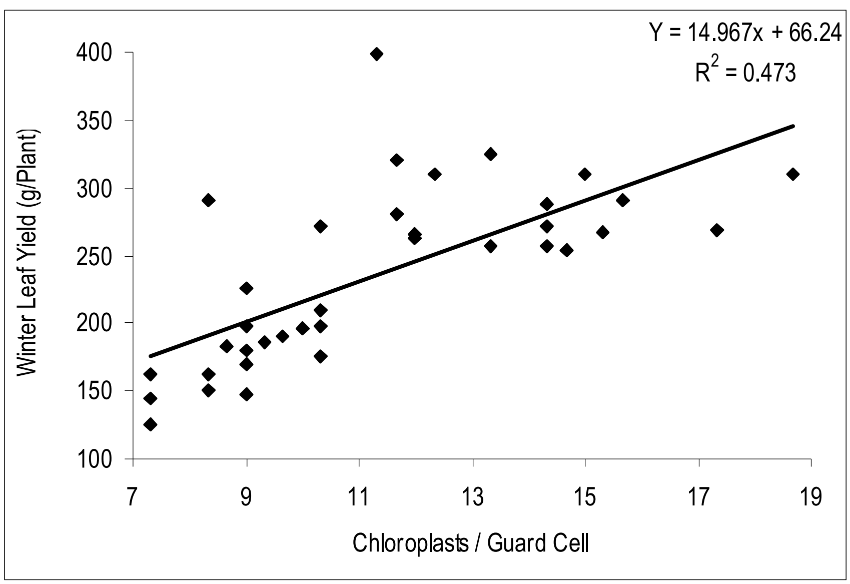
Parameters	**Winter leaf yield**	**Days to sprout**
Electrical conductivity (dS/m)	0.74**	-0.73**
Net photosynthetic rate (μmol $m^{-2}s^{-1}$)	0.82**	-0.53**
Nitrate reductase activity (μ mol NO_2^- h^{-1} g^{-1} fr. Wt)	0.90**	-0.58**
No. of chloroplast/guard cell	0.68**	-0.48**

** Significant at 1% level.

Table 3. Details of genotypes identified as parents and grouped on the basis of EC values

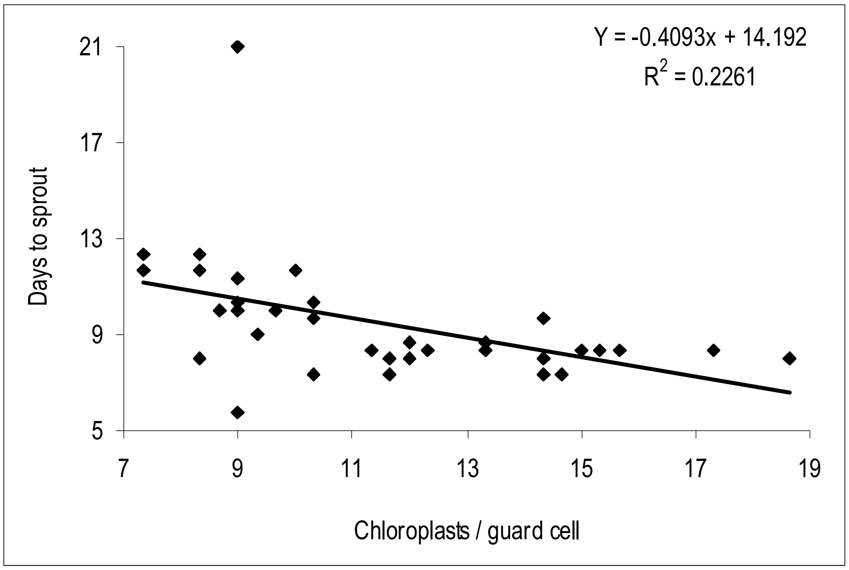
Category	**Sex**	
	Male Parent	Female Parent
High EC=(>$\bar{x}$+SD) to ($\bar{x}$ +2SD)	Shrim-2, KPG-1.	*M.australis*, Sujanpur-5, Kolitha-9, T-27, *M.induca* HP and Kolitha-8
Medium EC=($\bar{x}$) to ($\bar{x}$ +SD)	Mandalaya, Kosen and C-2028.	CSRS-II, Nagaland local, Philippines, Kolitha-7, C-2038, T-2, T-13 and T-22.
Low EC=(<$\bar{x}$)	Thailand unlobed, Italian mulberry, Monla, Berhampore-B and V-1.	Punjab local, Sultanpur, Kolitha-3, Matigara black, Dudhia red, Berhampore-A, Cyprus, Kanva-2, Kajli (OP), T-7, T-21 and Kajli.

Fig. 1



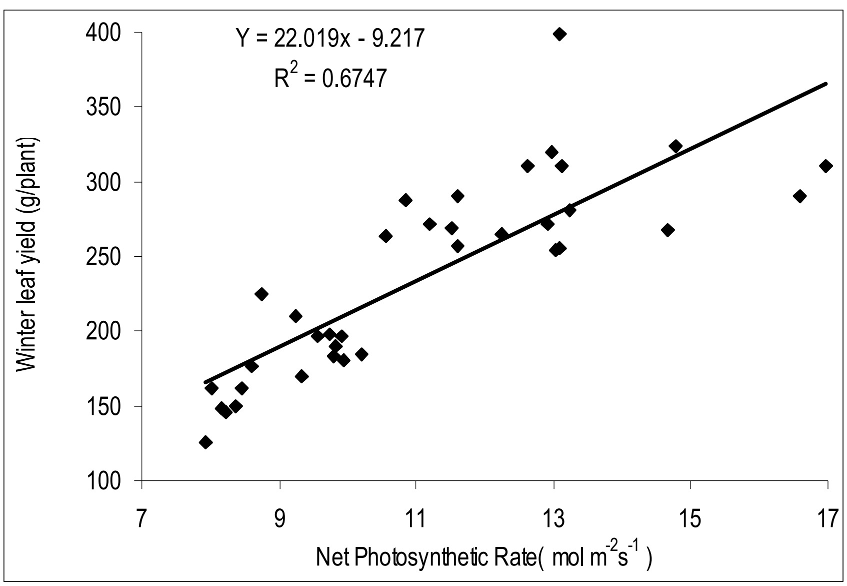
Regression and correlation coefficients between winter leaf yield / plant and number of chloroplasts /guard cell.

Fig. 2



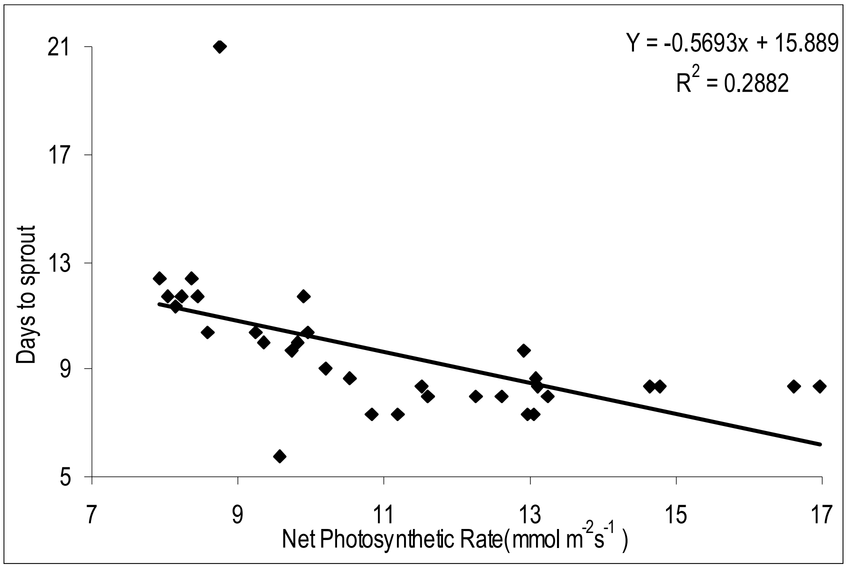
Regression and correlation coefficients between days to sprout after pruning and number of chloroplasts /guard cell

Fig. 3



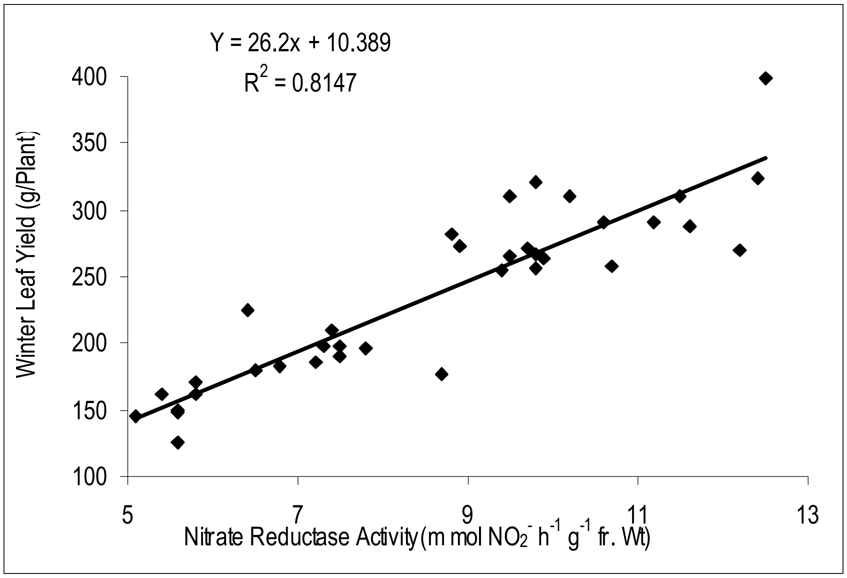
Regression and correlation coefficients between winter leaf yield / plant and net photosynthetic rate

Fig. 4



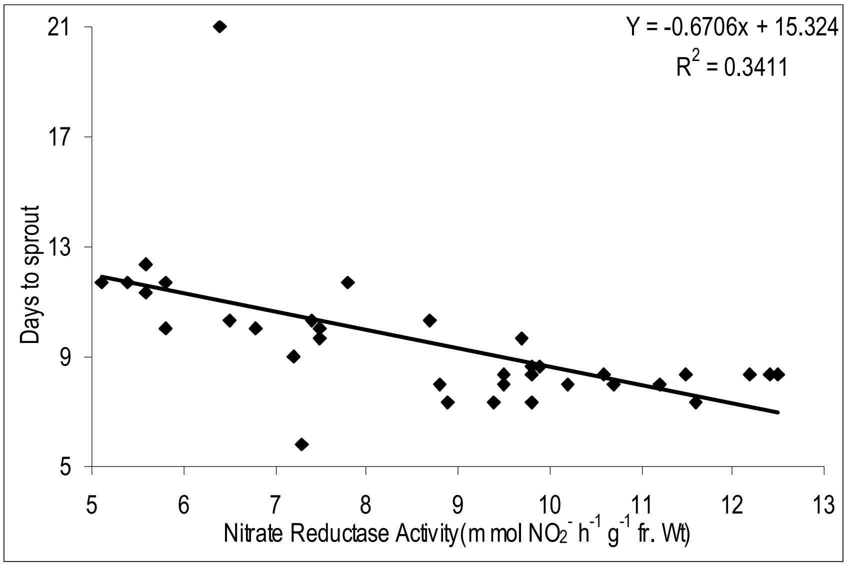
Regression and correlation coefficients between days to sprout after pruning and net photosynthetic rate

Fig. 5



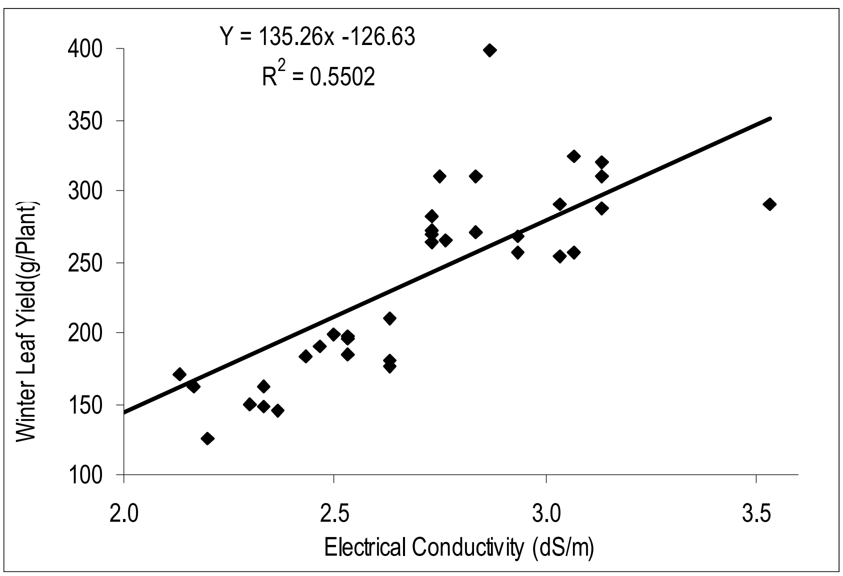
Regression and correlation coefficients between winter leaf yield / plant and nitrate reductase activity

Fig. 6



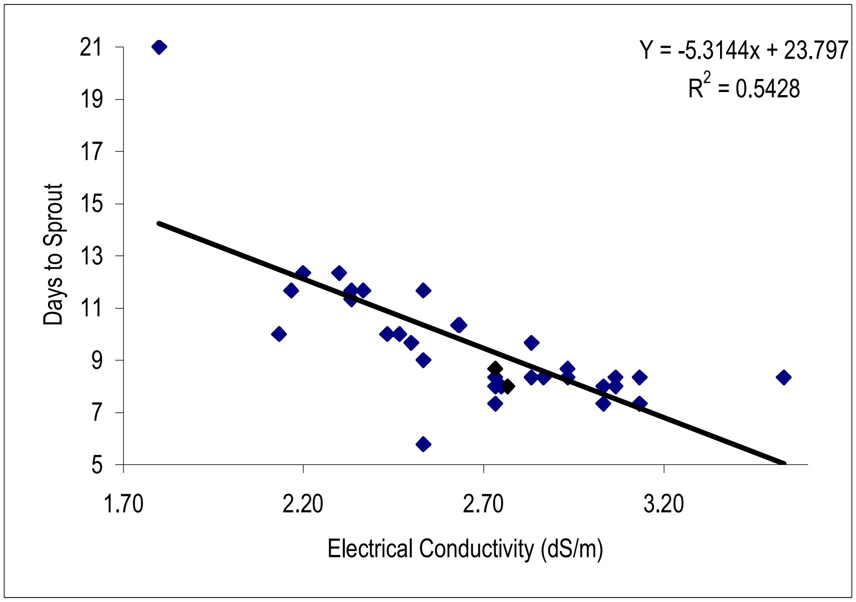
Regression and correlation coefficients between days to sprout and nitrate reductase activity

Fig. 7



Regression and correlation coefficients between winter leaf yield / plant and electrical conductivity of leaf sap

Fig. 8



Regression and correlation coefficients between days to sprout after pruning and electrical conductivity of leaf sap

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9/8/2012