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Effect of low temperature stress on betel vine (*Piper betle* L.) types, *Bangla* and *Desavari*

Rajesh Tripathi, Garima Khare & Nikhil Kumar[1]

Betel Vine Laboratory
National Botanical Research Institute
Lucknow - 226 001, India.

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Abstract

The effect of low temperature stress was studied in two types (*Bangla* and *Desavari*) of betel vine (*Piper betle*). Among the two types, *Desavari* showed better performance under cold stress and had significantly higher leaf thickness, levels of total thiols and peroxidase activity and lower lipid peroxidation.

Key words : betel vine, cold stress and *Piper betle*.

Betel vine (*Piper betle* L.), a shade-loving climber and native of milder climate, often suffers due to cold temperatures prevalent in North India during winters. Cultivation of betel vine in this region could become possible with the development ingeneous technology of fully protected cultivation some 2000 years ago (Kumar 1999). Cold stress leads to cessation of growth, tip drying, loss of chlorophyll, drying of leaf margins and sometimes the whole vine and the extent of damage may range from 15% to total crop failure (Anon 1997). Such a high degree of sensitivity to cold makes it an unique system for characterization of cold stress responses. The present investigation reports some of the preliminary findings on cold stress responses in two betel types, *Bangla* and *Desavari*.

The experimental plants were grown in the Betel Vine Conservatory at Banthra Research Station of National Botanical Research Institute, about 25 km from Lucknow (India). Leaf samples were collected during January and February (1999) when cold injury symptoms are well manifested. The leaf samples with or without marked symptoms of cold damage were analysed using standard methods. Leaf water status was estimated by measuring the relative water content (Irigoven *et al.* 1992). Free proline was estimated using ninhydrin (Larher *et al.* 1993) and total thiol by Ellman's method (Ellman 1959). Lipid peroxidation was estimated by extracting leaf samples in trichloro acetic acid (TCA) using thiobarbituric acid reagent (Dhindsa & Matowe 1981) and the colour was read at 535 nm. Subtracting the absorbance at 600 nm made correction for non-specific absorbance. Peroxidase activity was assayed (Toivonen & Sweeny 1996) using guaicol and H_2O_2 system. Increase in absorbance due to oxidation of guaicol was read at 470 nm. The ability of leaves to fix CO_2 was estimated by measuring CO_2 dependent (ca. 5% CO_2 in the system) O_2 evolution in leaf discs in Hansatech oxygen electrode (Walmer 1987).

Cold stress leads to water deficit in the leaf. Betel vine leaves with visible injury showed greater water deficits compared to the ones devoid of it. In *Bangla*, the water deficit was more as compared to that of *Desavari*, which was reflected in their Relative Water Content. In *Desavari*, the extent of water deficit in damaged and undamaged leaf was less possibly due to greater leaf thickness. Cold stress in many cases (Bangall *et al.* 1983; Wilson 1976; Eamus 1987) is known to cause water deficit. One of the earliest visible symptoms of low temperature stress in chilling sensitive plants is wilting. This is due to high transpiration

[1] For correspondence

rates that is effected by locking-open or slowing the stomatal closure, associated with reduced water supply from roots or impediments in translocation in the xylem vessels due to cold stress. Greater leaf thickness in *Desavari* may be one of the factors for better water retention and may also account for better performance of the type (Table 1 a & b).

Proline accumulation (Larher *et al.* 1993) is considered to be one of the major and ubiquitous stress response. In cold damaged leaves, the level of proline was more when compared to leaves without any injury. Difference in proline levels were also seen between the types; however, the differences were non significant. Many stress factors cause damage by increasing the overall oxidative stress (Prasad *et al.* 1994). Low temperature stress, in case of sensitive plants makes it vulnerable to photoinhibition causing further damage to the plant (Powles 1984). Photosynthetic activity reflecting photosynthetic capacity under saturating CO_2 concentration measured as CO_2 dependent O_2 evolution was adversely affected due to cold stress and in *Bangla* the differences were significant. Lipid peroxidation in cold injured leaves was more as compared to undamaged leaves and it was more so in the *Bangla* type as compared to *Desavari*. Non-protein thiols represent the general redox status of the plants (De Kok & Oosterhuis 1983). From the data it appears that damaged leaves accumulated more thiols in both the types. However, the type showing better adaptation also showed overall higher levels of thiols. As a consequence of cold stress there is a build-up of peroxide levels in the system (Prasad *et al.* 1994), which are known to be toxic. Peroxidases play an important role in the detoxication of peroxides in system. Total peroxidase activity in the crude extract was higher in *Desavari* than *Bangla* type. Thus the detoxifying system against reactive oxygen buildup appears to be more effective in *Desavari* and also partly explains the differences in lipid peroxidation (Table 1 a & b).

This preliminary study suggests that the betel type *Desavari* is relatively better adapted to cold stress than *Bangla* due to certain structural and biochemical features. Detailed studies would be needed to understand the behavior of different types to cold stress. This may also help in selection

Table 1a. Effect of cold stress on *Piper betle* types *Bangla* and *Desavari*

Parameter	*Bangla*			*Desavari*		
	Damaged	Undamaged	% Change over Undamaged	Damaged	Undamaged	% Change over Undamaged
Relative water content (%)	90.90 ± 6.52	97.50 ± 1.11	6.74	96.65 ± 2.36	98.58 ± 0.96	1.96
Leaf thickness (μm)	-	237 ± 05		-	275 ± 06	
Proline (n mol g^{-1})	10.94 ± 2.07	10.57 ± 0.73	3.50	10.76 ± 1.81	9.31 ± 1.49	14.93
Lipid peroxidation (m mol g^{-1})	13.63 ± 1.50	10.57 ± 2.35	28.95	11.48 ± 0.77	6.08 ± 1.80	88.82
Thiol (n mol g^{-1})	150.0 ± 34.0	89.0 ± 7.0	68.54	195.0 ± 3.0	159.0 ± 1.0	22.64
Peroxidase (katal)	2.29 ± 0.01	1.50 ± 0.06	52.66	3.48 ± 0.22	3.03 ± 0.14	14.85
Oxygen evolution (μ mol m^{-2} s^{-1})	1.33 ± 0.46	3.66 ± 0.75	63.66	3.18 ± 0.28	3.69 ± 0.58	13.82

- Not determined
Values are mean ± SD of 6 observations for biochemical assays and 20 leaf discs for RWC and leaf thickness

Table 1b. Calculated t values of different combination of means

Parameter	T_1	T_2	T_3	T_4
Relative water content	4.72**	3.18**	2.84*	3.53**
Leaf thickness			6.33**	
Proline	0.46	1.52	1.97	1.61
Lipid peroxidation	2.75	7.25**	3.75**	3.27**
Thiol	20.32**	31.18**	30.31**	24.36**
Peroxidase	39.09**	4.33**	26.50**	17.18**
Oxygen evolution	6.62**	2.04	0.07	8.66**

** Significant at 1%; *Significant at 5%

T_1 Undamaged *Bangla* and damaged *Bangla*; T_2 Undamaged *Desavari* and damaged *Desavari*; T_3 Undamaged *Bangla* and undamaged *Desavari*; T_4 Damaged *Bangla* and damaged Desavari

of types better suited for cultivation in the subtropics where the plants are under fully protected cultivation.

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