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EFFECTS OF HALOPRIMING ON PHOTOCHEMISTRY OF VIGNA RADIATA (L.) WILCZEK SUBJECTED TO OSMOTIC STRESS

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The investigation was carried out to study the effect of halopriming on photochemistry of three *Vigna radiata* varieties subjected to NaCl/PEG stress. The changes in the photochemistry of *V. radiata* seedlings were monitored by measuring the total chlorophyll content, PS I and II activities, chlorophyll *a* fluorescence related parameters like density of active reaction centres (RC/CS), absorbtion of photons (ABS/CS), trapping of photons (TR/CS), electron transport (ET/CS) and dissipation of energy (DI/CS) per cross section of PS II. The application of stress resulted in the retardation of growth, total chlorophyll content, photosystem I and II activities and changes in the chlorophyll a fluorescence parameters of the seedlings. It can be concluded that halopriming treatments of the seeds could decrease the extent of reduction in the photosynthetic efficiency of the seedlings of three *V. radiata* varieties subjected to NaCl/PEG stress and it was evident in the NaCl tolerant variety Pusa Vishal.

Keywords: Halopriming; osmotic stress; photochemistry; photosystem activity; chlorophyll

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Green gram (Vigna radiata (L) Wilczek) is a tropical legume which is an important dietary pulse crop of India enriched with lysine and protein. Various abiotic stresses, especially drought and salinity are the major limitations to its production. Seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. For priming the seeds, the seed is either soaked in water (hydropriming), solutions of poly ethylene glycol (PEG) (osmopriming), salt (CaCl2, CaSO4, NaCl, etc.) or some specific chemicals prior to germination (Patade et al., 2009). The seedlings emerging from primed seeds showed early and uniform germination. In halopriming with NaCl, the seeds were treated in NaCl concentrations of tolerable limits (Jisha et al., 2013).

In the present study, the effects of halopriming on the photochemistry of the seedlings were studied by analyzing the photosynthetic pigment content, photosystem (PS) I, II activities and chlorophyll a fluorescence related parameters.

Materials and Methods

The seeds of V. radiata varieties (Pusa Ratna- abiotic stress sensitive, Pusa 9531- drought tolerant, Pusa Vishal- NaCl tolerant) were obtained from seed science and technology division, IARI, New Delhi. The seeds were soaked in NaCl solutions for 6 h and were later dried back to their original moisture content. The priming concentration of NaCl (35 mM for Pusa Ratna, 50 mM for Pusa 9531 and 75 mM for Pusa Vishal) and the time period (6 h) of priming was determined based on the preliminary screening studies. Primed as well as non-primed seeds were germinated in plastic bottles (19×11 cm) containing absorbent cotton soaked with distilled water (control), different concentration of NaCl and PEG solution. The stress imparting concentrations of NaCl and PEG were also determined through a series of standardization process. Chlorophyll estimation was carried out by the method of Arnon (1949).

Thylakoids from leaves were isolated according to Puthur (2000) and the photochemical activities of the isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) at 40C as per the protocol of Puthur (2000). The activity of Photosystem I (PS I) and Photosystem II (PS II) was expressed in terms of nmol of O2 consumed/evolved min-1 mg-1 chlorophyll. Chlorophyll a fluorescence related parameters were monitored by using a Handy PEA (Hansatech, UK) according to Strasser et al. (2004).

Results

Leaf Pigment Composition

As a result of NaCl/PEG stress, photosynthetic pigment content of the seedlings was decreased in all the three varieties and the halopriming of seeds decreased the extent of this reduction. In general, the tolerant varieties (Pusa 9531 and Pusa Vishal) showed more response towards halopriming treatments by showing prominent increase in the photosynthetic pigment content of leaves when compared to non-primed controls. While the abiotic stress sensitive variety Pusa Ratna, was unable to produce such a prominent increase in chlorophyll a and b content of the seedlings subjected to unstressed and stressed (NaCl/PEG). Carotenoid content was also increased in all the three V. radiata varieties which were raised from haloprimed seeds. Among the three varieties, maximum increase in carotenoid content was recorded in the Pusa 9531 seedlings which were grown under PEG stress (78%) (Table 1).

Activity of Photosystems

The reduction in the PS I and II activities of seedlings as a result of NaCl/PEG stress was partially

Table 1: Photosynthetic pigment content of leaves of V. radiata seedlings under halopriming and exposed to 0, NaCl and PEG-6000 stress. The data is an average of recordings from three independent experiments each with three replicates (i.e. n=9). The data represent mean+standard error.

Varieties	*Chlorophyll a (mg/g dw)*			*Chlorophyll b (mg/g dw)*		
	0 stress	**NaCl stress**	**PEG stress**	**0 stress**	**NaCl stress**	**PEG stress**
Pusa Ratna	18.78±0.86 (16.51±0.95)	12.00±0.76 (12.00±0.83)	9.00±0.36 (9.00±0.45)	5.99±0.21 (5.15±0.35)	2.65±0.11 (3.03±0.21)	2.81±0.08 (2.24±0.01)
Pusa 9531	18.51±0.56 (15.98±0.13)	15.17±0.85 (12.72±0.65)	5.23±0.13 (3.23±0.12)	6.29±0.42 (5.09±0.32)	4.50±0.32 (4.02±0.34)	1.41±0.01 (0.94±0.01)
Pusa Vishal	24.20±1.06 (20.82±0.95)	9.96±0.44 6.36±0.83)	11.55±0.84 9.16±0.54)	7.76±0.31 (6.31±0.21)	2.64±0.09 (1.62±0.01)	3.64±0.09 2.46±0.02)
Varieties	*Total chlorophyll (mg/g dw)*			*Carotenoids (mg/g dw)*		
	0 stress	**NaCl stress**	**PEG stress**	**0 stress**	**NaCl stress**	**PEG stress**
Pusa Ratna	24.77±1.66 (21.69±1.34)	14.51±0.55 (15.01±0.63)	11.12±0.55 (12.44±0.31)	8.69±0.42 (6.79±0.31)	5.14±0.32 (5.40±0.21)	3.72±0.11 (4.07±0.13)
Pusa 9531	24.74±1.57 (21.03±1.06)	19.64±0.75 (16.65±0.65)	8.33±0.44 (4.17±0.12)	7.61±0.31 (6.41±0.22)	6.02±0.22 (5.25±0.08)	2.63±0.12 (1.48±0.06)
Pusa Vishal	31.93±1.24 (27.03±1.04)	12.96±0.14 (7.96±0.23)	18.72±0.45 (11.59±0.54)	9.97±0.42 (7.78±0.31)	4.46±0.11 (3.36±0.09)	4.65±0.07 (4.14±0.08)

**The values in the parenthesis denote the value of photosynthetic pigments in leaves of the seedlings of three V. radiata varieties raised from non-primed seeds exposed to 0, NaCl and PEG-6000 stress.*

alleviated in the seedlings raised from haloprimed seeds. In general, on halopriming, the tolerant varieties showed maximum enhancement in the PS I activities of seedlings when they were subjected to the stresses for which they are known to be tolerant. Among the three varieties studied, halopriming resulted in four fold increase of PS I activity in the seedlings of NaCl tolerant variety Pusa Vishal especially under NaCl stress condition. The drought tolerant variety Pusa 9531 along with Pusa Ratna recorded high percentage of increase in PS I activity of seedlings when the seedlings were subjected to PEG stress (84 and 182%, respectively) (Figure 1A). As far as the PS II activity was concerned, Pusa Vishal showed maximum enhancement in the PS II activity of seedlings which were raised from halorpimed seeds and subjected to unstressed and stressed (NaCl/PEG) conditions and it was 76, 72 and 60%, respectively (Figure 1B).

grown under unstressed conditions. The halopriming treatments of seeds showed increase in various phenomenological flux ratios such as RC/CS, ABS/CS, TR/CS and ET/CS while decreased the DI/CS of PS II reaction centers in all the three varieties of V. radiata seedlings. In all the three varieties studied, it was found that the seedlings subjected to stressed (NaCl/PEG) conditions showed more response to the halopriming treatments when compared to those subjected to unstressed conditions by showing better results in the chlorophyll a fluorescence related parameters (Figures 2, 3, 4).

Discussion

The observed reduction in chlorophyll content under NaCl/PEG stress may be due to the degradation of chlorophyll pigments or interference in the synthesis of chlorophyll. Ashraf and Rasul (1988) reported that the reduction in chlorophyll content under osmotic stress is due to the

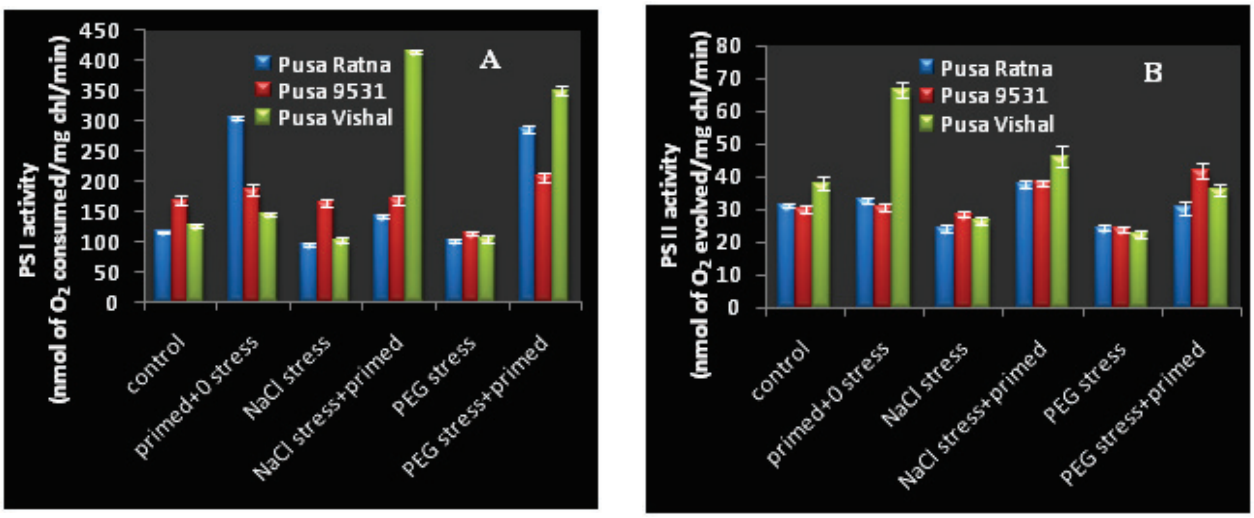

Figure 1: Photosystem (PS) I (A) and II (B) activity of the seedlings of three V. radiata varieties raised from haloprimed seeds and grown under unstressed and stressed (NaCl/PEG) conditions. The vertical bars represent SE of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates.

Chlorophyll *a* Fluorescence Related Parameters

As a result of NaCl/PEG stress, the phenomenological flux ratios RC/CS, ABS/CS, TR/CS and ET/CS decreased in the seedlings of V. radiata as compared to those seedlings which were grown under unstressed conditions. Whereas the dissipation of energy (DI/CS) was found to be increased in the seedlings which were subjected to stress conditions as compared to the seedlings

suppression of enzymes required for chlorophyll synthesis. From the results it was clear that halopriming improved the photosynthetic efficiency in all the three varieties and it was significant in the NaCl tolerant variety Pusa Vishal which was evident from the high chlorophyll content, high PS I and PS II activities in the primed seedlings. On halopriming, the rate of chlorophyll degradation was not to the extent as that observed in the case of non-primed

seedlings. Therefore, the energy harvested by the chlorophyll would have been effectively channelized into the reaction centres, resulting in an enhanced PS I and PS II activities (Jisha *et al*., 2014).

The Chlorophyll *a* fluorescence analysis of photochemistry is a fast, non-invasive, non-destructive and very useful tool to study physiological state of photosynthetic apparatus of any photosynthesizing material. It is the re-emission of light energy absorbed by chlorophyll a molecules or re-emitted red/far red light (mostly from PSII). The leaf models deals with the phenomenological energy fluxes (per cross-section). A decrease in density of active reaction centers (RC/CS) were observed in the seedlings which was subjected to stressed conditions. It is due to the conversion of active centers into inactive centers and is evident from the leaf models. The decrease in the energy absorbed per excited cross-section (ABS/CS) in the seedlings subjected to stress conditions indicates that the energy absorption efficiency of PS II was decreased under stressed conditions. From the leaf models it was also clear that the trapping of energy (TR/CS) and electron transport (ET/CS) was also reduced under stressed conditions in the seedlings of *V. radiata*. The electron transport reduction under stressed conditions is mainly due to reduced number of active reaction centers occurring under stress. All the three seed priming treatments caused an increase in the RC/CS, ABS/CS, TR/CS and ET/CS, which indicates the positive influence of seed priming. It implies that the seed priming treatments enhanced the count of open reaction centers and also increased the efficiency of absorption, trapping and transport of electrons per PS II cross section. Seed priming treatments also decreased the

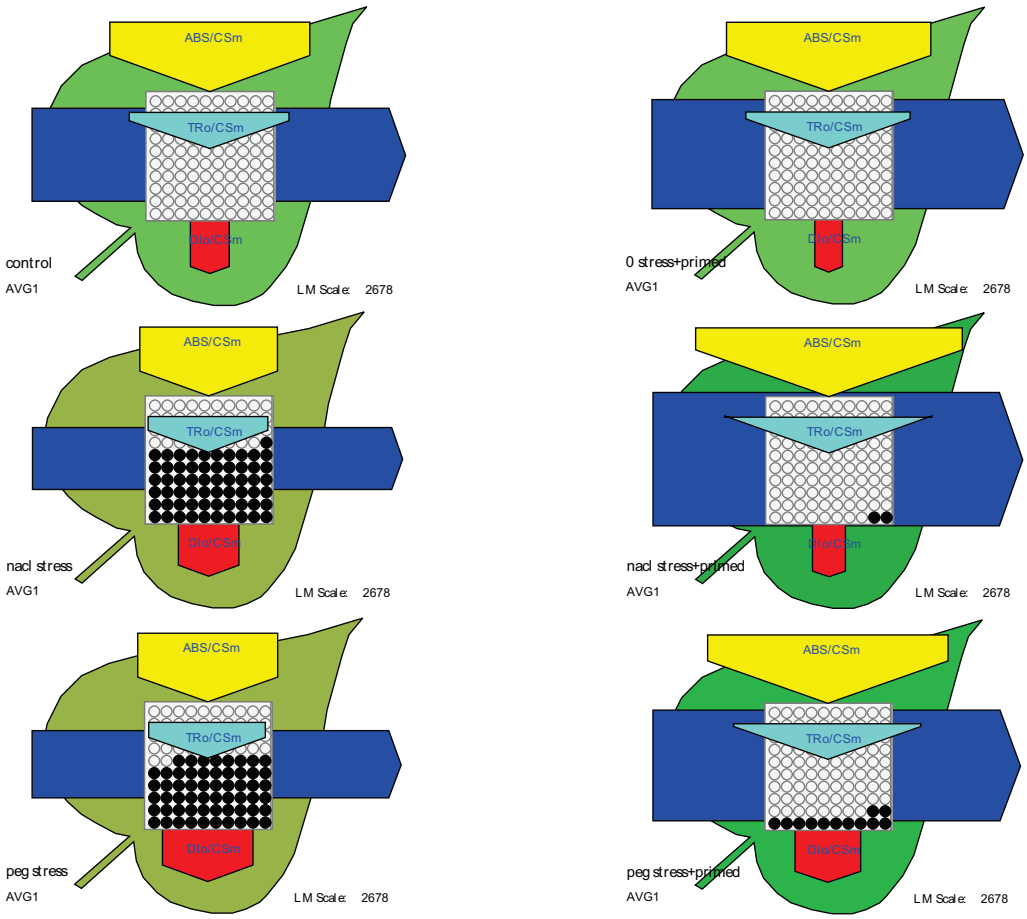

Figure 2: Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in V. radiata variety Pusa Ratna subjected to halopriming treatments: The value of each parameter can be seen in relative changes in width of each arrow. Active RCs are shown as open circles and inactive RCs are shown as closed circles.

dissipation of energy thus maximizing the efficiency of reaction centers by effectively trapping the energy and further utilizing it.

CONCLUSION

From the present investigation it can be concluded that the halopriming of seeds effectively reduced the deleterious effects of NaCl/PEG stress in all the varieties of *V. radiata* especially in the NaCl tolerant variety Pusa Vishal as analysed from data related to photosystem (I and II) activity and chlorophyll *a* fluorescence.

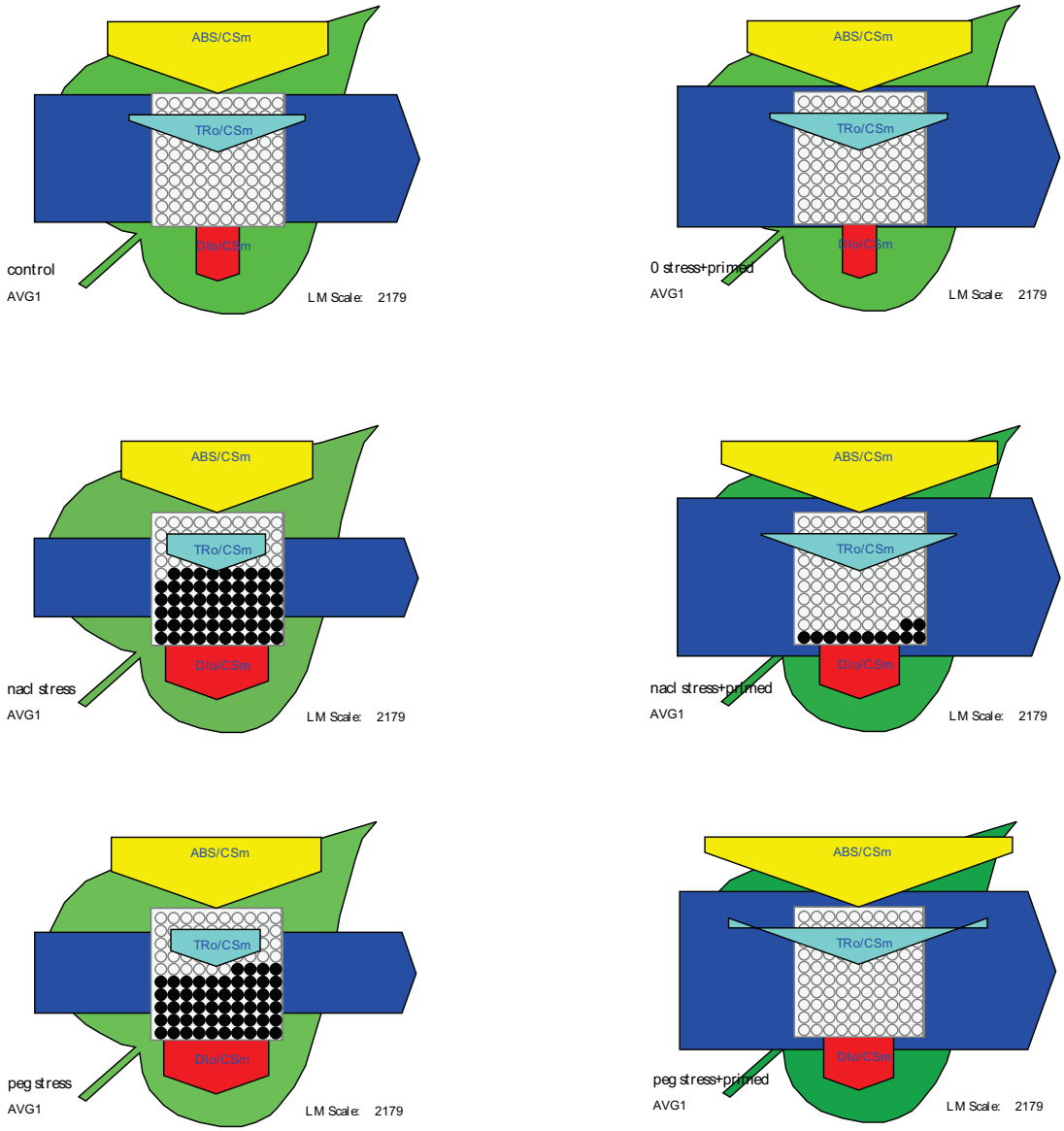

Figure 3: Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in V. radiata variety Pusa 9531 subjected to halopriming treatments: The value of each parameter can be seen in relative changes in width of each arrow. Active RCs are shown as open circles and inactive RCs are shown as closed circles.

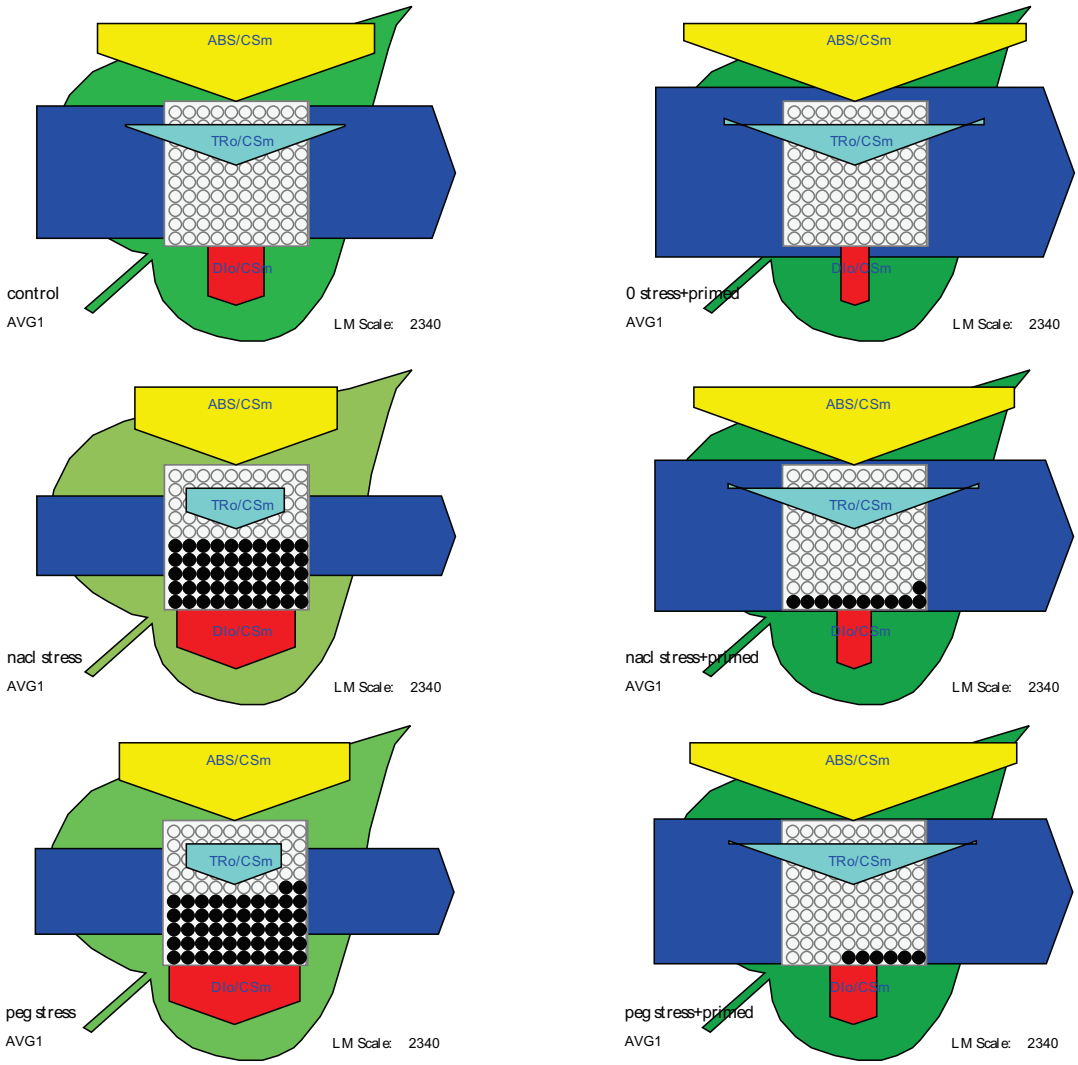

Figure 4: Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in V. radiata variety Pusa Vishal subjected to different halopriming treatments: The value of each parameter can be seen in relative changes in width of each arrow. Active RCs are shown as open circles and inactive RCs are shown as closed circles.

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