

Short Communication

Division of Floriculture, IARI, N. Delhi, India

Chlorophyll and Proline as Affected by Moisture Stress in Young and Mature Leaf Tissues of *Brassica carinata* Hybrids and Their Parents

S. R. Voleti, V. P. Singh and D. C. Uprety

Authors' addresses: Dr V. P. Singh and Dr D. C. Uprety, Division of Plant Physiology, Dr S. R. Voleti, Division of Floriculture and Landscaping, Indian Agricultural Research Institute, N. Delhi -12, India

With 2 tables and 2 figures

Received March 19, 1997; accepted May 27, 1997

Abstract

Chlorophyll reduction and proline accumulation under moisture stress at three different growth stages in young and mature leaf tissues of *B. carinata* hybrids of reciprocal origin and their parents was studied. Greater chlorophyll reduction was observed in mature leaves at flowering stage, whereas proline accumulated significantly in young leaves at pre-flowering stage. The chlorophyll and proline contents *per se* in hybrids was higher so as their reduction and accumulation compared to their parents. Maternal influence was apparent for these two characters in *Brassicas*.

Key words: Chlorophyll — proline — growth stage — young leaf — mature leaf — hybrids and parents

Introduction

Retention of chlorophylls and proline accumulation have been reported as stress related adaptations in several crop species, including *Brassicas* (Ashraf and Mehmood 1991, Mukherjee et al. 1991). Chlorophylls to some extent inherit to offspring/hybrids maternally because of active involvement of cell autonomous bodies, i.e. chloroplasts. Recently, Jones et al. (1996), Voleti and Uprety (1997) and Uprety et al. (1990) have reported maternal influence on kernel sink capacity in maize and leaf photosynthesis in *Brassicas*. Often, the stress responses were studied in the mature tissues and the information on young tissues at different growth stages is rather scarce. In this communication, we have examined the chlorophyll and proline levels from young (still expanding) and mature leaf (expanded but not yet yellow) tissues of *Brassica* hybrids of reciprocal origin and their parents under the influence of moisture stress.

Materials and Methods

The tetraploid hybrids, *B. carinata* 226 (with *B. nigra* cytoplasm), *B. carinata* 241 (with *B. oleracea* cytoplasm) (Prakash et al. 1984) naturally occurring *B. carinata* and their parents were grown in earthen pots with 12 kg air dried soil under the natural environment. Moisture stress treatment was given at pre-flowering (30 days after sowing, DAS), flowering (60 DAS) and post-flowering (90 DAS) by withholding irrigation till the leaves showed wilting symptoms. When the symptoms begun, samples from young (still expanding upper most) and mature (middle region of the plant) were taken in triplicates for chlorophyll and proline estimations. Chlorophylls were extracted using dimethyl sulfoxide and the absorbance was read on a Bausch and Lomb spectrophotometer at wavelengths 665 and 649 nm. Total chlorophylls were calculated using the equations reported by Barnes et al. (1992). Proline extraction was done in sulfosalicylic acid as reported by Bates et al. (1972). The data is statistically analysed for genotype, position, treatment and their interaction.

Results

The tetraploid, *B. carinata* hybrids differed significantly in their response to moisture stress treatment. Maximum chlorophyll reduction was observed in younger leaves of *B. carinata natural* (43 %), *B. carinata* 241 (38 %) at pre-flowering stage, whereas in the parents reduction was more at post-flowering stage (32 and 22 % in *B. oleracea* and *B. nigra*, respectively). On the other hand, the mature leaves in *B. carinata* 226 had greater reduction followed by *B. carinata natural* (38 %). Both the parents exhibited greater reduction of chlorophylls at post-flowering stage (Table 1).

Similar to chlorophylls, the young leaf tissues of *B. carinata natural* (41 %) and *B. carinata* 241 (39 %)

Table 1: Influence of moisture stress on chlorophyll content (mg g^{-1} fr. wt.) at different stages in *Brassica* genotypes

| Genotypes | Pre-flowering | | Flowering | | Post-flowering | |
|----------------------------------|---------------|--------|-----------|--------|----------------|--------|
| | Control | Stress | Control | Stress | Control | Stress |
| Young leaf | | | | | | |
| <i>Brassica carinata</i> natural | 2.54 | 1.45 | 2.41 | 2.49 | 1.80 | 1.40 |
| <i>Brassica carinata</i> 226 | 1.62 | 1.56 | 3.32 | 2.53 | 1.62 | 1.31 |
| <i>Brassica nigra</i> | 1.69 | 1.43 | 3.05 | 2.50 | 1.51 | 1.17 |
| <i>Brassica carinata</i> 241 | 2.02 | 1.25 | 2.79 | 1.98 | 1.20 | 0.84 |
| <i>Brassica oleracea</i> | 1.30 | 1.21 | 3.07 | 2.32 | 0.68 | 0.46 |
| Mean | 1.83 | 1.39 | 2.93 | 2.36 | 1.56 | 1.04 |
| Mature leaf | | | | | | |
| <i>Brassica carinata</i> natural | 2.59 | 2.27 | 4.41 | 2.74 | 1.74 | 1.96 |
| <i>Brassica carinata</i> 226 | 2.07 | 1.40 | 5.33 | 3.26 | 2.57 | 2.25 |
| <i>Brassica nigra</i> | 2.31 | 2.06 | 3.54 | 2.79 | 2.43 | 1.90 |
| <i>Brassica carinata</i> 241 | 2.26 | 2.04 | 2.89 | 2.01 | 1.81 | 1.11 |
| <i>Brassica oleracea</i> | 2.22 | 1.88 | 2.46 | 1.98 | 1.16 | 0.84 |
| Mean | 2.29 | 1.93 | 3.73 | 2.56 | 1.94 | 1.61 |
| CD at 5% | | | | | | |
| Genotype (G) | 0.150 | | 0.062 | | 0.030 | |
| Position (P) | 0.095 | | 0.039 | | 0.019 | |
| Treatment (T) | 0.095 | | 0.039 | | 0.019 | |
| G \times P | 0.212 | | 0.088 | | 0.042 | |
| G \times T | 0.212 | | 0.088 | | 0.042 | |
| P \times T | NS | | 0.056 | | NS | |
| G \times P \times T | 0.299 | | 0.124 | | 0.060 | |

exhibited greater proline accumulation than the mature leaf tissues at pre-flowering stage. The parents had greater proline accumulation at pre-flowering stage, even greater than hybrids. Consistently, the mature tissues had greater proline at flowering stage, except *B. oleracea* (Table 2).

Irrespective of genotypes, the younger leaves had greater chlorophyll reduction and proline accumulation at post-flowering and pre-flowering stages

(41 %), respectively, whereas the mature leaves at flowering stage (29 and 31 % for chlorophyll and proline, respectively).

Similarly, irrespective of position, the reduction in chlorophyll was greater at flowering stage (26 %) while proline was greater at pre-flowering (31 %) stage (Figs 1 and 2).

On the other hand, the reduction of chlorophylls were higher during post-flowering stage (33 %)

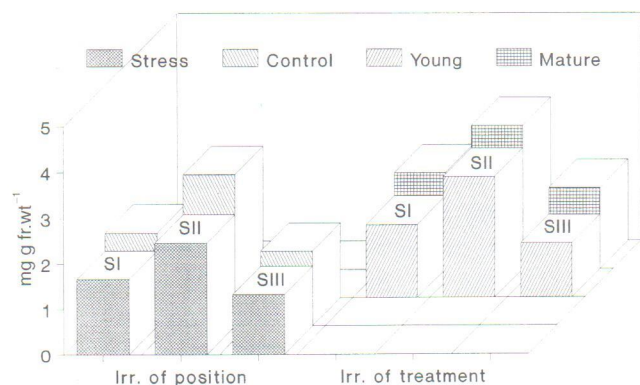
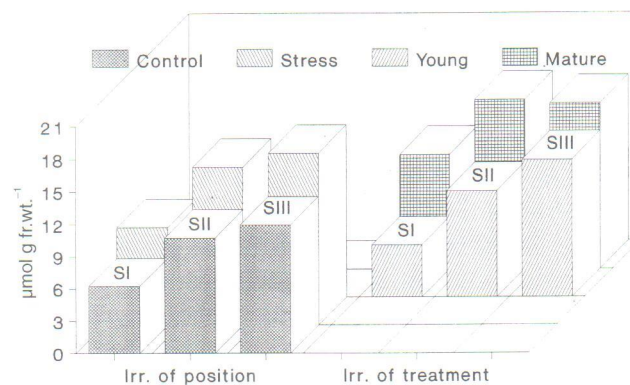
Fig. 1: Chlorophyll content under moisture stress in *Brassica* genotypesFig. 2: Proline accumulation under moisture stress in *Brassica* genotypes

Table 2: Influence of moisture stress on proline content ($\mu\text{mol g}^{-1}$ fr. wt.) at different stages in *Brassica* genotypes

| Genotypes | Pre-flowering | | Flowering | | Post-flowering | |
|----------------------------------|---------------|--------|-----------|--------|----------------|--------|
| | Control | Stress | Control | Stress | Control | Stress |
| Young leaf | | | | | | |
| <i>Brassica carinata</i> natural | 3.48 | 5.95 | 8.68 | 11.95 | 12.16 | 17.03 |
| <i>Brassica carinata</i> 226 | 4.01 | 5.43 | 6.91 | 7.76 | 10.11 | 14.71 |
| <i>Brassica nigra</i> | 2.44 | 4.16 | 6.68 | 8.64 | 9.16 | 13.74 |
| <i>Brassica carinata</i> 241 | 2.29 | 3.77 | 9.50 | 11.64 | 10.64 | 12.40 |
| <i>Brassica oleracea</i> | 5.83 | 11.09 | 11.23 | 15.18 | 10.93 | 16.06 |
| Mean | 3.61 | 6.08 | 8.60 | 11.03 | 10.60 | 14.79 |
| Mature leaf | | | | | | |
| <i>Brassica carinata</i> natural | 11.11 | 14.70 | 11.79 | 18.56 | 12.96 | 16.90 |
| <i>Brassica carinata</i> 226 | 8.12 | 10.11 | 13.58 | 17.98 | 13.57 | 16.82 |
| <i>Brassica nigra</i> | 6.79 | 8.65 | 11.45 | 16.46 | 12.59 | 13.77 |
| <i>Brassica carinata</i> 241 | 6.65 | 9.00 | 9.45 | 13.37 | 13.43 | 13.98 |
| <i>Brassica oleracea</i> | 11.70 | 18.25 | 18.18 | 24.93 | 14.26 | 23.93 |
| Mean | 8.87 | 12.14 | 12.89 | 18.26 | 13.36 | 17.08 |
| CD at 5% | | | | | | |
| Genotype (G) | 0.90 | | 1.07 | | 0.90 | |
| Position (P) | 0.57 | | 0.68 | | 0.57 | |
| Treatment (T) | 0.57 | | 0.68 | | 0.57 | |
| G \times P | 1.27 | | 1.52 | | 1.28 | |
| G \times T | 1.27 | | NS | | 1.28 | |
| P \times T | NS | | 0.96 | | NS | |
| G \times P \times T | NS | | NS | | 1.81 | |

while proline accumulated to a greater level at pre-flowering stage (54 %) based on irrespective of treatment.

Discussion

Selection of the tissues to visualize stress responses is of prime significance to derive valid conclusions of susceptible/tolerance nature of a given species. Further, the complexity of the nature is compounded by the expression of genes at the time of necessity. Therefore, the stress responses needs to be analysed critically through different growth stages. In the present study, it is clear from Tables 1 and 2 that *B. carinata* hybrids of reciprocal origin had shown differential responses to stress treatment. In general, the losses in chlorophyll content were higher in *B. carinata* natural and *B. carinata* 241 at pre-flowering stage, particularly in the younger tissues. As a coincidence, proline accumulation too was greater at this stage in the younger leaf tissues for these two genotypes. The role of proline in determining drought tolerance has been shown in barley (Naidu et al. 1992) and also in *Brassicas* (Mukherjee et al. 1991). In the present case it appears that the

younger leaf tissues are somehow being protected by increase in their proline content as a protective mechanism. The percentage decrease in chlorophyll content was lower in the parents compared to the hybrids. However, the differences in the chlorophyll content *per se* between the parents and hybrids was much larger. These differences in chlorophyll content could be because of change in ploidy level as was shown in panicum (Wullschelegar et al. 1996) and *Brassicas* (Upreti et al. 1990). It was interesting to note that the hybrids resembled that of their female parents in the behaviour of the degradation in their chlorophyll content. Generally, the mature leaves of hybrids had greater chlorophyll reduction at flowering stage. On the other hand, whether it was younger or mature leaf tissues, both were found equally susceptible for chlorophyll degradation at post-flowering stage. The post-flowering stage reduction in chlorophyll was often associated with age. Proline, which plays a significant role in combating stress accumulated to different degrees. Among the hybrids *B. carinata* 241 and *B. carinata* natural accumulated greater proline content in younger leaves whereas *B. carinata* 226 in mature

leaves. Parents, on the other hand, were found to accumulate more proline in younger and also in mature tissues (Tables 1 and 2). One of the parent, *B. oleracea* had greater proline content *per se* and also accumulated more proline. This was in contrast with chlorophyll degradation. As an adaptive phenomenon proline might have accumulated to a greater degree in the younger tissues since, it can act as osmoticum as in the case of barley (Naidu et al. 1992).

The greater chlorophyll reduction in the mature tissues compared to the young and comparatively greater proline accumulation in younger tissues have a positive significant role in drought tolerance mechanisms in that the younger tissues are protected through the manipulation of the proline accumulating capability. The variation of proline within the *Brassica* genotypes may be once again due to the ploidy levels. Interestingly, like chlorophylls, proline accumulation of hybrids too resembled that of maternal parents. So far, no evidence with regard to maternal influence on proline had been shown. However, the key enzymes of proline metabolism were reported to be present in chloroplast (Stewart 1981) hence, such a possibility cannot be ruled out.

To summarize, in response to stress loss in chlorophyll occurred in mature tissues at flowering stage, whereas proline accumulated in younger leaves at pre-flowering stage. Hybrids were found to be superior in drought tolerance nature due to the higher initial content and closely resembled to that of their parents in the reduction of chlorophylls and proline accumulation capacity.

Zusammenfassung

Chlorophyll und Prolin unter dem Einfluß von Feuchtigkeitsstress in jungen und reifen Blattgeweben von *Brassica carinata*-Hybriden und ihren Eltern

Eine Chlorophyllreduktion und Prolinakkumulation unter Feuchtigkeitsstress in drei unterschiedlichen Wachstumsstadien von jungen und reifen Blattgeweben

von *B. carinata*-Hybriden von reziprokem Ursprung und ihren Eltern wurde untersucht. Eine stärkere Chlorophyllreduktion wurde in reifen Blättern in der Blühphase beobachtet, während Prolin signifikant in jungen Blättern zur Vorblühphase akkumulierte. Chlorophyll- und Prolingehalte *per se* in Hybriden waren, sowie deren Reduktion und Akkumulation im Vergleich zu ihren Eltern erhöht. Der mütterliche Einfluß war für diese beiden Eigenschaften bei *Brassica* deutlich.

Acknowledgements

Our thanks to Dr S. Prakash for providing the genetic material.

References

- Ashraf, M., and S. Mehmood, 1990: Response of four *Brassica* species to drought stress. *J. Exp. Bot.* **30**, 93—100.
- Bates, L. S., R. P. Waldren, and I. D. Teare, 1973: Rapid determination of free proline in water stress studies. *Plant and Soil* **39**, 205—207.
- Jones, R. J., M. W. B. Scheeiber, and J. A. Roessler, 1996: Kernel sink capacity in maize. Genotypic and maternal regulation. *Crop Sci.* **36**, 301—306.
- Mukherjee, S.P., S. N. Banerjee, S. Gupta, and B. B. Mukherjee, 1991: Effect of water, salt and freezing stress on *B. juncea* (L.) czern callus. *Ind. J. Exp. Biol* **29**, 182—183.
- Prakash, S., S. Gupta, R. N. Raut, and A. Kalra, 1984: Synthetic *Brassica carinata* — a preliminary report. *Cruciferae news lett.* **9**, 36.
- Stewart, C. R., 1981: Proline accumulation: biochemical aspects. In: L. G. Paleg and D. Aspinall (eds.), *The physiology and Biochemistry of drought resistance in plants*, pp. 243—259. Academic Press Sydney.
- Upreti, D. C., S. Prakash, and V. K. Tomar, 1990: Cytoplasm influences the photosynthetic efficiency in *Brassica carinata*. *J. Agron. Crop. Sci.* **165**, 207—210.
- Voleti, S. R., and D. C. Upreti, 1997: Photosynthetic characteristics in *Brassica carinata* hybrids and their parents as influenced by moisture stress. *Biol. Plantarum* (in press).
- Wullschlegar, S. D., M. A. Sanderson, S. B. McLaughlin, D. P. Biroda, and A. C. Royburn, 1996: Photosynthetic rates and ploidy levels among populations of switch grass. *Crop Sci.* **36**, 306—312.