BRIEF COMMUNICATION

# Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings

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## Abstract

With the increase in concentration of applied salicylic acid (SA), chlorophyll (Chl) content decreased significantly in both wheat and moong seedlings. Chl *a/b* ratio decreased significantly only in wheat and remained constant in moong. On the other hand, total carotenoid (Car) content, size of xanthophyll pool, and de-epoxidation rate increased significantly with an increase in SA concentration in both plant species. Hence SA treatment may induce Car biosynthesis in these plant species, but the increase in the xanthophyll pool and de-epoxidation rate indicates that SA may create oxidative stress the degree of which is different in various plants.

*Additional key words*: oxidative stress; *Triticum*; *Vigna*; xanthophyll pool.

According to Raskin (1992), salicylic acid (SA) should be included in the category of phytohormones. Relatively little work has been done on the influence of this compound on plant metabolism. SA plays an important role in flower induction, growth and development, ethylene biosynthesis, stomatal behaviour, and respiration (Raskin 1992). It is important in disease resistance (Raskin 1992, Klessig and Malamy 1994) but the exact mode of action of SA in this direction is not known. SA establishes systemic acquired resistance by inducing pathogenesis-related genes (Bi *et al.* 1995, Conrath *et al.* 1995) *via* reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$) (Chen *et al.* 1993). SA inhibits the activities of catalase and ascorbate peroxidase and hence the content of $H_2O_2$ increases (Chen *et al.* 1993, Durner and Klessig 1995, Rao *et al.* 1997, Kawano and Muto 2000). The carotenoids (Car) protect photosynthetic apparatus from ROS, which are generated under stress (Siefermann-Harms 1987, Panda and Biswal 1989, Srichandan *et al.* 1989, Demmig-Adams 1990, Young 1991). We investigated the influence of SA on the contents of chlorophyll (Chl) and Car in wheat (monocot) and moong (dicot).

Seeds of wheat (*Triticum aestivum* L. cv. Raj-1555) and moong (*Vigna radiata* cv. Pusa Baishakhi) were washed in distilled water and sterilised for 2 min by immersion in 0.1 % $HgCl_2$ solution. After a careful washing with distilled water they germinated in Petri dishes on filter paper in different concentrations of salicylic acid (0, 5, 10, 50, 100, and 200 mg $kg^{-1}$). The seedlings were grown for 120 h in growth chambers (*Nippon Medical & Chemical Instruments*) at an irradiance of 300 µmol $m^{-2}$ $s^{-1}$ and a temperature of 25 °C with the light/dark cycle of 12/12 h. The experiment was replicated three times.

Pigments were extracted and quantified according to the modified method of Gilmore and Yamamoto (1991). Pigments were extracted in dim light by grinding the frozen samples in the mortar with 100 % acetone. The extract was centrifuged at 240 rps for 3 min, and supernatant was used for Chl and Car separation which was performed with HPLC (*Shimadzu*).

The total Chl (*a*+*b*) content decreased significantly in wheat with an increase in the SA concentration (except for 10 mg $kg^{-1}$) (Fig. 1*A*). In moong, Chl content was lower in control plants than in SA-treated ones and it decreased significantly with an increase in SA concentration. A reduction in Chl content in barley leaves following the application of SA was found by Pancheva *et al.* (1996). Foliar application of SA also caused a decline

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*Abbreviations*: A – antheraxanthin; Car – carotenoid; Chl – chlorophyll; ROS – reactive oxygen species; SA – salicylic acid; V – violaxanthin; VAZ – xanthophyll pool; Z – zeaxanthin.

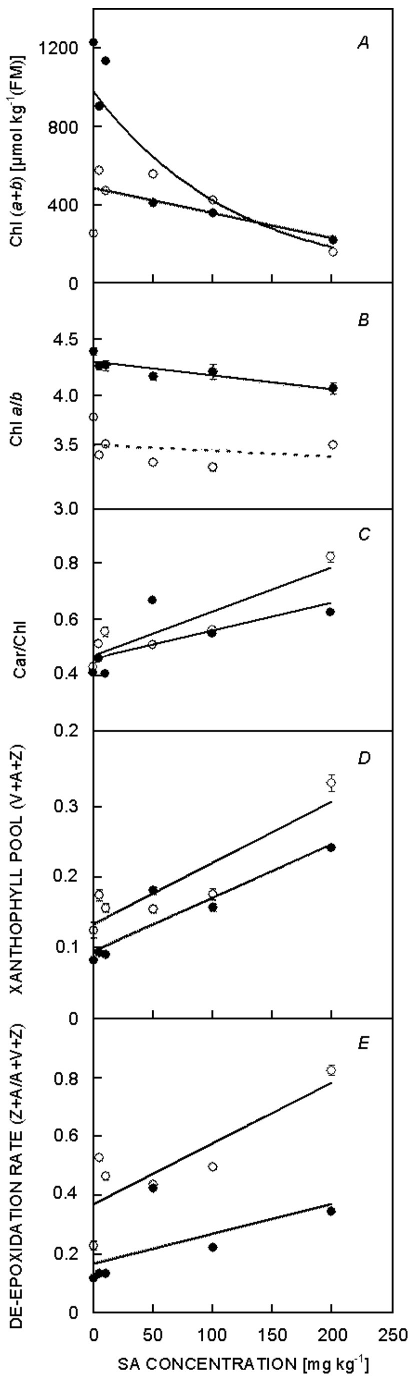


Fig. 1. Effects of different concentrations of salicylic acid on the chlorophyll (Chl) (*a*+*b*) content of wheat ($r^2$ = 0.85, $p$ = 0.0000) and moong ($r^2$ = 0.34, $p$ = 0.001) seedlings (*A*), the Chl *a/b* ratio of wheat ($r^2$ = 0.33, $p$ = 0.0115) and moong ($r^2$ = 0.072, $p$ = 0.3096) seedlings (*B*), total carotenoid (Car) content of wheat ($r^2$ = 0.47, $p$ = 0.0015) and moong ($r^2$ = 0.77, $p$ = 0.0000) seedlings (*C*), the xanthophyll pool of wheat ($r^2$ = *0.85*, $p$ = 0.0000) and moong ($r^2$ = 0.75, $p$ = 0.0000) seedlings (*D*), and de-epoxidation (Z+A)/(V+A+Z) rate of wheat ($r^2$ = 0.38, $p$ = 0.0066) and moong ($r^2$ = 0.67, $p$ = 0.0000) seedlings (*E*). Wheat (●) or moong (○), average of $n$ = 3 for each SA concentration treatment.

in Chl content of *Vigna mungo* (Anandhi and Ramanujam 1997). In wheat Chl *a/b* ratio decreased significantly with an increase in SA concentration (Fig. 1*B*), indicating that SA affected light-harvesting antenna size. However, in moong it remained constant. The change in the Chl *a/b* ratio is used as an indicator for relative photosystem stoichiometry (Pfannschmidt *et al.* 1999). Chl *a/b* ratio was comparatively higher in wheat than in moong, suggesting that the light-harvesting antenna size was smaller in wheat than in moong.

In contrast to Chl, the content of total Car increased significantly with an increase in SA concentration in both crops (Fig. 1*C*). Plant growth regulators variously influence Car accumulation. In etiolated seedlings of wheat and maize, Šesták and Ullmann (1960) noted a decrease in Car content after gibberellic acid treatment. Sunderland (1966) noted partial inhibition of Car synthesis by 2,4-D in tissue cultures of *Oxalis dispar* and *Haplopappus*. They further noticed that naphthalene acetic acid also caused 2,4-D like effects. A 30 % inhibition of Car biosynthesis by abscisic acid in excised fragments of etiolated maize leaves was found by Mercer and Pughe (1969). In contrast to the above plant growth regulators, in *Arabidopsis thaliana* leaves 1 mM SA caused an increase in Car content, whereas 5 mM SA caused a decrease in the Car content in comparison with the con-trol (Rao *et al.* 1997). In our experiment, an increase in SA concentration stimulated Car accumulation in both crops (Fig. 1*C*).

SA induces an increase in hydrogen peroxide ($H_2O_2$) content in plants *via* inhibition of catalase and ascorbate peroxidase (Chen *et al.* 1993, Durner and Klessig 1995, Rao *et al.* 1997, Wendehenne *et al.* 1998, Kawano and Muto 2000, Luo *et al.* 2001). In higher plants, Car protects the photosynthetic apparatus from excess photons and oxidative stress (Siefermann-Harms 1987, Panda and Biswal 1989, Srichandan *et al.* 1989, Demmig-Adams 1990, Young 1991). In our experiments, the significant increase in total Car and decrease in total Chl content with an increase in SA concentration might be of some protective value. An increase in oxidative stress may cause decrease in total Chl content or a decrease in total Chl content might induce oxidative stress with an increase in SA concentration. Similarly, the total Chl content was higher in wheat than in moong and total Car content was higher in moong than in wheat (Fig. 1*A*,*B*), indicating that lower Chl content in moong might induce greater oxidative stress than in wheat. To protect the photosynthetic apparatus from the oxidative stress, the level of Car might be increased (Fig. 1*C*).

The size of xanthophyll pool (VAZ) and de-epoxidation rate also increased significantly with an increase in SA concentration (Fig. 1*D,E*). An increase in total Car and VAZ may partly offer photoprotection to Chl (Fig. 1*C,D*). Under stress, zeaxanthin (Z) and possibly antheraxanthin (A) are responsible for the quenching of excess excitation energy (Gilmore and Yamamoto 1993). VAZ determines the capacity for zeaxanthin formation (Gilmore and Yamamoto 1993). The size of VAZ increases under stress, *e.g.* high irradiance (Demmig-Adams *et al.* 1989, 1995, Logan *et al.* 1996). In our study also VAZ increased with an increase in SA concentration in both crops and it was higher in moong than in wheat (except for 50 mg $kg^{-1}$) (Fig. 1*D*). The enzymatic de-epoxidation of violaxanthin (V) to Z *via* A [de-epoxidation rate is (Z + A)/(V + A + Z)] was also considerably increased with an increase in SA concentration in both crops (Fig. 1*E*). Hence the Z content increased with an increase in SA concentration in both crops. De-epoxidation rate was higher in moong than wheat. By quenching the excited Chl singlet state, Z protects photosynthetic apparatus from photoinhibition (Demmig-Adams 1990).

Our results suggest that an increase in SA concentration may induce oxidative stress in both wheat and moong but the degree of oxidative stress is different in different plant species.

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