Effect of indol-3-yl acetic acid on photosynthetic characteristics of wheat flag leaf during grain filling

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Abstract

Area and fresh and dry masses of flag leaf show two phases of development during grain filling in Triticum aestivum. The initial large increase in leaf size is mainly due to water intake. Contents of chlorophylls and carotenoids, reducing sugars, and sucrose, Hill reaction rate, and photosynthetic activity increased during leaf growth, but a noticeable decline in these parameters followed throughout leaf senescence. The maximum accumulation of polysaccharides and proteins occurred at the beginning of grain set, but a continuous decline in their absolute values was manifested during grain filling. Grain priming with indol-3-yl acetic acid (IAA) at 25 mg kg\(^{-1}\) stimulated the flag leaf growth, namely its fresh and dry masses and its area. Furthermore, the stimulatory effect was mainly due to the increase in the pigment formation that in turn increased the photosynthetic activity of flag leaf during grain filling. On the other hand, the highest dose of IAA (50 mg kg\(^{-1}\)) attenuated the growth and physiological activity of flag leaf through its inhibitory action on leaf fresh and dry masses, leaf area, pigments, saccharides and protein formation, as well as its effect on \(^{14}\)CO\(_2\) assimilation.

Additional key words: carotenoids; chlorophylls; \(^{14}\)CO\(_2\) assimilation; flag leaf; fresh and dry masses; Hill reaction; leaf area; photosynthates; polysaccharides; proteins; sugars.

Introduction

Efficient translocation of photoassimilates to growing seeds is one of the key factors increasing seed production (Yamagata et al. 1987). The main source of carbon for grain filling in modern wheat is that produced by current photosynthesis in flag leaf (Austin andEdrich 1975, Aldesuquy 1990, Wenshan et al. 1994). When \(^{14}\)CO\(_2\) is fed to flag leaf of an intact plant, only a minor proportion of the \(^{14}\)C fixed is transferred to parts of tillers (Rawson and Hofstra 1969), but the major proportion of photosynthates is transported to spikes (Wenshan et al. 1994). The importance of leaf area in controlling plant matter and growth rate was confirmed by Potter and Jones (1977) who concluded that rates of leaf area expansion have a greater influence on dry matter production than does net assimilation rate.

Growth regulators are important for leaf development, maturation, and senescence (Lovesys et al. 1987). They are involved in yield capacity and distribution of assimilates between panicle and shoot (Herzog 1982).

Both synthetic and natural auxins can delay senescence in tissues (Baker 1983, Lyons and Widmer 1984). In Lilium longiflorum, application of IAA at 100 or 200 mg kg\(^{-1}\) increased plant height and the number of leaves per plant (Pal and Das 1990). The number of mango leaves and total leaf area per plant increased with increasing IAA concentration up to 1000 mg kg\(^{-1}\) (Narwadkar and Anserwadekar 1989). Translocation of \(^{14}\)C-photo synthates was increased by application of IAA to spikes but decreased by abscisic acid (ABA) application (Wenshan et al. 1994). IAA treatment at 0.05\% prolonged the life of the most productive beet leaves (10-25) by 5 d and increased leaf area and photosynthetic potential (Arkhangelskii et al. 1999).

The flag leaf in cereal plants is a powerful source of assimilates translocated to developing grains. The objective of this study was to investigate the efficacy of IAA in improvement of photosynthetic characteristics of wheat flag leaf during grain filling.
Materials and methods

A field experiment was established in 1998 at the garden of the Faculty of Science, Mansoura University, Mansoura, Egypt. Homogeneous *Triticum aestivum* L. (cv. Giza 158) grains were surface sterilized by soaking in 0.01 M MgCl₂ solution for 3 min, washed thoroughly with distilled water, and divided into 4 sets. Grains of the 1st set were soaked in distilled water to serve as control, while grains of the 2nd, 3rd, or 4th sets were soaked in 5, 25, and 50 mg kg⁻¹ IAA solutions, respectively, for 8 h. After soaking, thoroughly washed grains were drilled on 25 October 1998, in singles in rows 30 cm apart using the Afi method of planting. At heading stage (after ear emergence), the crop received 35 g(N) m⁻² as ammonium nitrate and 35 g(P) m⁻² as superphosphate.

For estimation of leaf area and fresh and dry matter, ten samples were used, and for chemical analyses triplicate samples were taken at 0, 2, and 5 weeks post-anthesis. Leaf lamina area was measured as: 0.70×length×breadth (Quarrie and Jones 1979). Extraction, clarification, and determination of saccharides were carried out following the procedure of Younis et al. (1969). Protein content was determined according to Lowry et al. (1951). Contents of chlorophyll (Chl) a and b and carotenoids were estimated by the spectrophotometric method as recommended by Metzner et al. (1965).

For determining ¹⁴CO₂ uptake (Gaber 1985), 300 mg of leaf discs was introduced into the fixation apparatus (Fig. 1). Aqueous solution of ¹⁴C-sodium carbonate of known activity (3.7 MBq cm⁻²) was pipetted into the apparatus followed by 0.2 cm³ H₂SO₄ (10%). The evolved ¹⁴CO₂ passed over and radioactivity of the green leaf discs was measured using a Packard Scintillation Counter.

For Hill reaction assay, detached leaves were ground using a chilled blender, in 50 mM MgCl₂ and the resulting homogenate was suspended in 1 mM Na-Tricine, 10 mM NaCl, and 10 mM MgCl₂ solution and kept at -4.0 °C (Arnon 1949). Photosystem 2 activity was determined as the rate of 2.6-dichlorophenol indophenol (2,6-DCPIP) photoreduction (Trebst 1972) using a Spectronic 21D spectrophotometer.

![Diagram](image)

**Fig. 1.** ¹⁴CO₂-fixation apparatus. A: main container; B: lid; C: inner container (1 cm³ capacity); D, E: side arms.

The results were first subjected to an analysis of variance (ANOVA). If ANOVA showed a significant ($p<0.05$) effect, the least significant difference was used to compare treatments (Snedecor and Cochran 1976).

Results

**Leaf area and fresh and dry masses:** The maximum leaf area of control plants (53.5 cm² per leaf) was attained after the 2nd week post-anthesis. As leaf has undergone senescence, its area declined till the 5th week post-anthesis (Fig. 2A).

At the beginning of grain set till the 5th week post-anthesis, IAA at 25 mg kg⁻¹ increased ($p<0.05$) the leaf area of wheat flag leaf in comparison with control plants. On the other hand, 50 mg kg⁻¹ IAA decreased ($p<0.05$) the flag leaf area during the overall growth period (Fig. 2A).

A progressive increase in flag leaf fresh and dry masses during grain filling up to the 2nd week post-anthesis was followed by a marked decrease on the 5th week post-anthesis (Fig. 2B,C). As compared with control, grain pretreatment with IAA particularly at 5 or 25 mg kg⁻¹ caused significant increase ($p<0.05$) in fresh and dry masses of wheat flag leaf during the grain filling period (Fig. 2B,C).

**Pigment contents:** Chl ($a+b$) content of control flag leaf at the beginning of anthesis was 1.69 g kg⁻¹(f.m.) followed by a marked increase on the 2nd week post-anthesis, thereafter a noticeable decrease was manifested on the 5th week (at the senescence). The carotenoid content of flag leaf slightly decreased on the 2nd week post-anthesis, followed by a slight increase on the 5th week (Fig. 2D,E,F).

Presoaking of wheat grains in IAA at 25 mg kg⁻¹ induced pronounced increase ($p<0.05$) in pigment contents of wheat flag leaf during the overall growth period if compared with the untreated plants. On the other
hand, the highest dose of IAA (50 mg kg⁻¹) attenuated the pigment contents of wheat flag leaf during grain filling. Chls and carotenoids of wheat flag leaf were non-significantly affected by the lower dose of IAA (Fig. 2D, E, F).

Reducing sugars and sucrose as well as the polysaccharide content of wheat flag leaf during the filling period (Fig. 3A, B, C).

Proteins: There was a gradual decline in protein content of wheat flag leaf till the 5th week post-anthesis (Fig. 3D).

Saccharides: There was a gradual increase in the amount of reducing sugars and sucrose in wheat flag leaf till the 2nd week post-anthesis. This was followed by their massive decline on the 5th week post-anthesis (Fig. 3A, B). The maximum accumulation of polysaccharides was attained at the beginning of grain set, followed by a progressive decline till the 5th week post-anthesis (Fig. 3C).

Grain pretreatment with IAA at 25 mg kg⁻¹ induced a massive increase (p<0.05) in contents of reducing sugars and sucrose at the expense of polysaccharide content of wheat flag leaf during the grain filling. However, the highest dose of IAA resulted in a large decrease in

Fig. 2. Effect of grain presoaking in indol-3-yl acetic acid on leaf area (A), fresh (B) and dry (C) masses, and contents of chlorophyll (Chl) a (D) and b (E) and carotenoids (F) of wheat flag leaf during grain filling. Bars in a group labelled with the same letter are not significant as indicated by LSD (p<0.05).

Fig. 3. Effect of grain presoaking in indol-3-yl acetic acid on contents of saccharides [reducing sugars (A), sucrose (B), polysaccharides (C)], and proteins (D), and Hill reaction rate by chloroplasts (E) of wheat flag leaf during grain filling. Bars in a group labelled with the same letter are not significant as indicated by LSD (p<0.05).

Compared with control plants, IAA at 50 mg kg⁻¹ induced a marked decrease in protein content of wheat flag leaf during the overall growth period. The medium concentration of IAA caused a massive accumulation of
Discussion

The increase in flag leaf area in the first phase of development is attributed to expansion through water uptake as confirmed by the strong correlation between area and fresh matter (Viana and Metivier 1980, Aldesuquy 1990). Dry matter content is mainly the result of carbon import from older leaves (Thiane et al. 1959). Chloroplasts, assimilate translocation, and contents of endogenous cytokinins, IAA, and ABA were studied during leaf development in potato cv. Malachite and barley cv. Varde. Lower leaves had higher growth rates and photosynthetic rates, while upper leaves showed more rapid development of assimilate export. Changes in growth, photosynthesis, and translocation were closely related to the content of endogenous growth regulators (Kiselyova et al. 1999).

The stimulating effect of 5 or 25 mg kg⁻¹ IAA on flag leaf growth and its area may be due to IAA involvement directly in leaf expansion and development (Herzog 1982). These results are in conformity with the results of Narwadkar and Anserwadekar (1989) and Kholodova et al. (1993). However, the cumulative flag leaf area decreased significantly during the grain filling in response to IAA at 50 mg kg⁻¹. This concentration of IAA probably induces high ratios of mesophyll surface area to leaf area (Kalaj and Nalborczyk 1991, Aldesuquy and Gaber 1993).

Chl contents, Hill activity, and ¹⁴CO₂ fixation were formed during leaf growth and their maxima occurred on the 2nd week from anthesis, parallel with the maximum expansion of leaf. The results are in accord with those obtained in pea (Smillie and Krotkov 1959), potato (Mokronosov and Bagaudinova 1974), Theobroma cacao (Baker et al. 1975), wheat (Aldesuquy 1990), etc. The decline in contents of leaf Chls, Hill activity, and photosynthetic formation on the 5th week post-anthesis is due to flag leaf senescence. These changes are similar to those reported by Jenkins (1981) and Baka and Aldesuquy (1991).
Grain priming with IAA at 25 mg kg\(^{-1}\) enhanced the production of pigments during the development of wheat flag leaf which in turn increased the rate of transformation of soluble photosynthates to insoluble ones. Furthermore, Hill reaction rate in flag leaf increased in response to 25 mg kg\(^{-1}\) IAA. The promotive effect of IAA on photosynthetic activity of flag leaf resulted from the massive increase in leaf area as well as from pigment production caused by IAA. IAA may also increase the rate of transpiration by inducing stomatal opening, with a concomitant increase in transpiration and photosynthesis rates (Wittenbach 1983, Nawadkar and Ansarwadakar 1989). IAA may also directly affect the photosynthetic machinery by increasing the plastid biogenesis in flag leaf and subsequently increase the number of proplastids or newly developed chloroplasts (Aldesuquy and Baka 1998). In this connection, there are many reports on plant hormone stimulation of development of chloroplasts and the whole photosynthetic machinery (e.g., Turner and Bidwell 1965, Wild et al. 1981, Sakr 1985, Noorden and Leopold 1988, Baka and Aldesuquy 1991). The results are also in good harmony with those obtained by Buschmann and Lichtenthaler (1979), Kadioglu (1992), Wenshan et al. (1994), Nanda and Nanda (1995), Kadiri et al. (1997). IAA stimulated CO\(_2\) uptake in Helianthus annuus and soybean leaves (Bidwell et al. 1968), ATP synthesis and bicarbonate uptake in isolated chloroplasts (Tamas et al. 1974), and P\(_{700}\) and Hill activity (Buschmann and Lichtenthaler 1979).

The changes in reducing sugars and sucrose during flag leaf development are consistent with the changes in leaf area and Chl content as well as with \(^{14}\)CO\(_2\) fixation. The observed decline in polysaccharides content of wheat flag leaf from the beginning of anthesis till the 5\(^{th}\) week post-anthesis was probably due to their mobilization to the emerged spikes to serve as a source of assimilates for the developing grains. Polysaccharides mobilization includes hydrolysis to saccharose and monosaccharides and this may be the reason for the observed increase in contents of reducing sugars and sucrose of wheat flag leaf during that period. The application of IAA particularly at 25 mg kg\(^{-1}\) IAA induced a massive increase in reducing sugars and sucrose at the expense of polysaccharides. Thus IAA may increase the degradation of polysaccharides where there is an increase in reducing sugars and sucrose at the same stage as well as increase their export out of the flag leaf. In experiments of Wenshan et al. (1994), translocation of photosynthates from wheat flag leaf was increased by application of IAA to spikes but decreased by ABA application. A considerable increase was found in pigment content of some plants when sprayed with IAA, accompanied with an increase in saccharide synthesis and translocation (Ahmed et al. 1989, Shaddad and El-Tayeb 1990). Kiseleva et al. (1998) found that the high sink activity and fast dry matter production in barley panicle were related to IAA which determined photosynthetic demand and their transport, partitioning, and metabolism, particularly during milky ripeness stage.

Our results showed that the protein content of wheat flag leaf was markedly reduced from the beginning of grain set till its maturation. This may be due to low accumulation and partition of reduced N accumulated during vegetative growth and low relative contribution of nitrate assimilation and N redistribution during leaf development. Alternatively, there might be an increase in proteolytic activity at that stage (Reed et al. 1980). Grain priming with 5 or 50 mg kg\(^{-1}\) IAA caused a large reduction in protein content of wheat flag leaf. Hence IAA may increase the translocation rate of protein from leaf flag to the developing grains. Bejaoui (1985) showed that protein synthesis was activated by IAA treatment, and Ahmed et al. (1989) found that externally applied 50 mg kg\(^{-1}\) IAA as foliar spray on maize, bean, and cowpea shoot led to an increase in total N contents, mainly protein. Moreover, Garcia Hernandez et al. (1996) found that IAA causes an increase in polysome formation in etiolated pea plants and a concomitant recruitment of p40, an acidic protein, into polysomes. Because p40 staining is very similar to that for RNA, they conclude that IAA induces p40, an accessory protein for ribosomes that might play a role in plant growth and development.

Generally, when the flag leaf senesces, leaves lose chlorophylls, photosynthetic activity, saccharides, and soluble proteins with a consequence of decrease in metabolic activity. These results agree with findings of Feller and Erismann (1978). Furthermore, IAA application particularly at 25 mg kg\(^{-1}\) improves pigment production in the flag leaf by retaining the Chl till 5\(^{th}\) week post-anthesis. Therefore, the increase in \(^{14}\)CO\(_2\) fixation induced more rapid movement of assimilates from flag leaf to the developing grains and finally improved the yield capacity of wheat plants. Noorden and Leopold (1988) found that auxin alters senescence-related processes such as Chl loss, RNA synthesis and degradation, protein synthesis and degradation, wilting, membrane breakdown, and changes in the contents of several enzymes (some increase, some decrease) in ways that are consistent with a senescence-delaying effect. However, IAA at 50 mg kg\(^{-1}\) inhibited the growth of flag leaf and the overall biochemical and physiological changes. These results agree with those of Hathout et al. (1993) who found that IAA at 80 mg kg\(^{-1}\) inhibited tomato growth and development, accompanied by increases in the content and activity of an endogenous inhibitor (mainly unsaturated lactone) and low contents of cytokinins and gibberellins.
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