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Changes in Ascorbic Acid Content during Growth and Development of *Panicum miliaceum*

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K. K. NANDA AND M. S. TAYAL[1]
Department of Botany, Panjab University, Chandigarh, India

ABSTRACT

The present paper deals with changes in the ascorbic acid content of the shoot apex during its transformation from the vegetative to the reproductive state and its further development in *Panicum miliaceum* var. Samai Co. 1. Seedlings were exposed to 24-hour illumination, natural day, and 8 hours of illumination per day. Ascorbic acid was determined for the growing apex, stem, and leaf of the main shoot and for the individual branches produced on it at successive developmental stages.

A several-fold increase in ascorbic acid content of the growing apex of the main shoot of plants grown under 8-hour illumination synchronizes with its transformation from the vegetative to the reproductive state. In plants grown under natural day and continuous illumination, which continue to grow vegetatively, the ascorbic acid content remains at a low level. Moving these plants to 8-hour illumination results in floral initiation and an increase in the ascorbic acid content. The high level of ascorbic acid is maintained through the gametogenic and embryogenic phases, but decreases during seed formation. A rise in ascorbic acid content of branch apices always occurs later than that in the main shoot but the increase again coincides with floral initiation in the branch apex. The ascorbic acid contents of stem and leaf are not significantly affected by the photoperiod. Hence, a massive upsurge in ascorbic acid content synchronizes with the transformation of the vegetative branch and shoot apices to the reproductive state.

It has been shown that in wheat and other plants the levels of AA[2] and IAA are closely correlated with the transformation of the growing apex from the vegetative to the reproductive state and with the differentiation of spikelets and their constituent flowers (3, 4, 7-9). We undertook investigations on *Panicum miliaceum* to determine the AA content of the shoot apex and other parts of the main shoot and branches at different ontogenic stages.

MATERIALS AND METHODS

Pure line seeds of *Panicum miliaceum* var. Samai Co. 1 obtained from the Director, Agriculture Research Institute, Coimbatore, India, were sown in January in unglazed earthen pots (30 cm diameter) containing a 3:1 mixture of garden soil and well rotted farmyard manure. Fifty seeds were sown and thinned after germination to 10 healthy seedlings per pot. Watering was done on alternate days until the end of February and then every day up to the termination of the experiment. The plants were fertilized with 2 g of superphosphate and 1 g of potassium sulfate every month.

Photoperiodic Treatments. The pots were divided into two groups. One group of plants was grown under continuous illumination (LD) and the other was left under the natural day length (ND) obtaining at Chandigarh (11-12 hr). After a lapse of 58 days, the plants of each group were subdivided into two other groups which received the normal days from March to July (15-16 hr day length) or short days (SD) with 8 hr of daily illumination alternating with 16 hr of darkness.

For LD treatment, the natural daylight was supplemented with artificial illumination from two 200-w daylight fluorescent lamps. To receive this supplemental light, the plants were transferred every day at sunset to a well ventilated room. The electric lamps were 3 ft apart and were adjusted 4 ft above the plant top. For SD treatment, the normal daylight was cut down by screening the plants with thick canvas sheets supported on wooden pillars from 2.00 PM to 7.00 PM daily.

Changes in Ascorbic Acid Level. Determinations of the AA contents of the shoot apex, stem, and young leaves of the main shoot and branches were made at regular intervals. The apex with about 1 mm of stem represented the shoot apex during the formative phase. After its change to the reproductive state, it was represented by the entire differentiating inflorescence. After the spikelets had differentiated, however, only the upper 10 of these were used for analysis and the term shoot apex refers to them. Still later when the floral organs were differentiated, stamens, pistils, and accessory parts from the uppermost flower of each of these 10 spikelets were analyzed separately for their AA content. Only the means of the contents of all these parts are shown in the figures to avoid crowding of the graphs. During the final stages, AA contents of the 10 kernels were determined.

The determinations of AA were made by a photoelectric colorimeter method developed by Chinoy (3, 5) employing 2,6-dichlorophenol indophenol. The weighed plant material was crushed in ice cold CO_2-saturated H_2O and the extract was made to a definite volume. Three milliliters of the extract were mixed with an equal volume of buffered metaphosphoric acid at pH 3.6. A 2-ml aliquot of this solution was mixed with 5 ml of distilled H_2O and the turbidity produced was adjusted to zero with a photocolorimeter. Another 2-ml aliquot was then mixed with 5 ml of 2,6-dichlorophenol indophenol prepared by dissolving 10 mg in 200 ml of distilled H_2O at 80 C and the optical density was measured. The amount of AA present in 1 ml of the original extract was obtained by using the regression formula:

$$Y = 0.1103 - 0.14X$$

where Y = concentration of AA in mg and X = optical density.

From the contents in 1 ml of the extract, the AA content per g fresh wt was calculated as follows: free AA = $(A \times V)/w \times 1000$, where A = mg AA/1 mg of the original extract, V = total

[1] Present address: Department of Botany, D.A.V. College, Mazaffarnagar (U.P.), India.

[2] Abbreviations: AA: ascorbic acid; LD: continuous illumination; ND: natural day length; SD: 8-hr illumination per day.

volume of the original extract (ml) and W = weight of the plant sample (mg) taken for analysis.

Records were also maintained of the dates of emergence of the ears on the main shoot and on the individual branches. These dates are shown by arrows in the Figures.

RESULTS

The AA content of the apex of the main shoot for plants grown under the three photoperiods are shown in Figure 1. In all cases, the AA content decreases at first, reaching a minimum 70 days after sowing (12 days after treatment) in SD plants and 78 days after sowing in ND and LD plants. In ND and LD plants, the AA content remains at a very low level until the end of the experiment but in the shoot apex of SD plants a rapid increase is apparent from the 77th to the 88th day, after which AA decreases again. LD and ND plants remain vegetative until the termination of the experiment. Earing takes place only under SD and the time of earing is indicated in the Figure by an arrow. Hence, there is a correlation between the AA content of the shoot apex and its transformation from the vegetative to the reproductive state.

The AA contents of the shoot apices of the main shoot and of the two branches—one emerging from the lower and the other from the upper node of the SD, ND, and LD plants—are

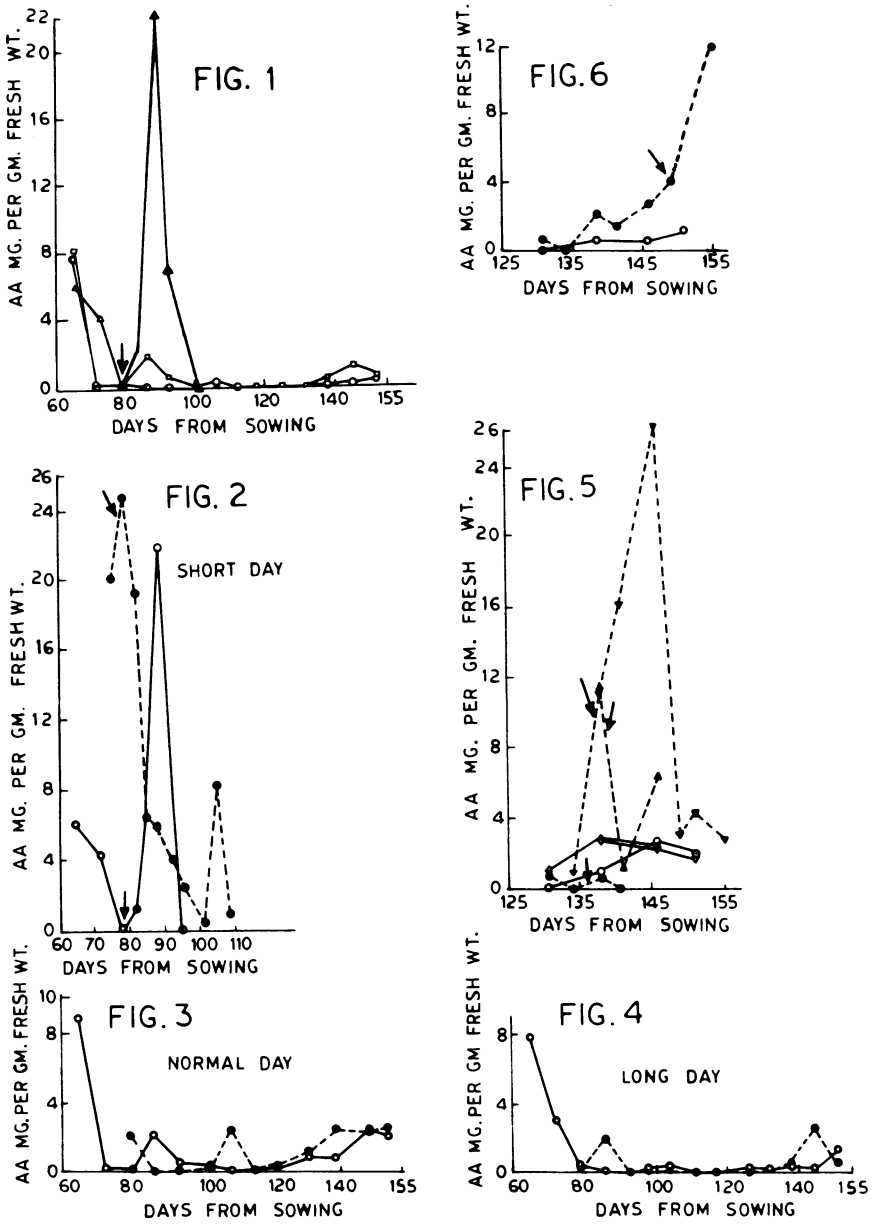

FIG. 1. Effect of Photoperoid on the AA content of the growing apex of the main shoot of *Panicum miliaceum*. SD (△); ND (□); LD (○). Arrow indicates the time of earing of SD plants.

FIG. 2. AA content of the growing apex of the main shoot (○) and one upper branch (●) of SD plant of *Panicum miliaceum*. Arrows indicate the time of earing of the main shoot and the branch.

FIGS. 3-4. AA content of the growing apex of the main shoot (○) and one lower branch (●) of ND (Fig. 3) and LD (Fig. 4) plant of *Panicum miliaceum*.

FIG. 5. AA content of the apex of the main shoot and one upper and one lower branch each of the ND-ND (▽, △, ○, respectively) and ND-SD (▼, ▲, ●, respectively) plants of *Panicum miliaceum*. Arrows indicate the time of earing of the main shoot and branches of ND-SD plants.

FIG. 6. AA content of the apex of the main shoots of LD-LD (○) and LD-SD (●) plants of *Panicum miliaceum*. Arrow indicates the time of earing of LD-SD plants.

presented separately in Figures 2 to 4. Those of the stem and leaf are not included in these figures to avoid crowding.

Figure 2 shows the AA contents in SD plants. We have already discussed the changes in AA content of the shoot apex of the main shoot. The contents of the stem and the leaf were high during the early stages, but in both cases declined to a low level after 78 days. As stated earlier, these have not been plotted to avoid crowding of Figures. The AA content of the shoot apex of the upper branch increases from 75 to 78 days and then gradually declines until the end, except during 102- to 105-day-period when they rise again. The first rise synchronizes with the transition of the shoot apex from the vegetative to the reproductive state and the second with the formation of a young kernel. No branch was produced from the lower nodes in SD plants.

The AA contents of the shoot apex of the main shoot as well as one of the lower branches, produced on ND and LD plants remain low throughout the period of experimentation (Figs. 3 and 4). In both these treatments, the shoot apex of the main shoot does not become reproductive, and no branch emerges from the upper nodes in LD plants. Even in ND plants, a branch that was initiated from the upper node showed a low level of AA.

ND and LD plants continued to grow vegetatively for 130 days, when they were subdivided into two groups to further confirm the relationship between the AA increase and the conversion of the growing apex to the reproductive state. While one group in each case continued to receive the same photoperiod, the other was transferred to short days. These plants are designated ND-SD and LD-SD, compared to ND-ND and LD-LD, which refer to those plants continuing under ND and LD conditions. Ear emergence was observed first in ND-SD plants and then in LD-SD ones, while ND-ND and LD-LD plants continued to grow vegetatively until the termination of the experiment.

The AA contents of the apex of the main shoot and the branches of ND-ND and ND-SD plants are shown in Figure 5. The contents of the growing apex of the main shoot as well as the branches of ND-ND plants remain low. In ND-SD plants, however, the AA contents of the shoot apex of the main shoot increase until the 146th day and decline after that. The AA content of the shoot apex of the uppermost and lowermost branches also increase from 134 to 146 days, whereas those of the branch next to the topmost increase from 142 to 146 days. This correlates with ear emergence which, as is indicated by the arrows is observed first in the main shoot, then in the uppermost shoot, and finally in the shoot in the lower branch.

Similar data with respect to LD-LD plants are presented in Figure 6. The AA contents of the shoot apex of the main shoot and branches of LD-LD plants remain low throughout the period of experimentation. These do not flower at all. But there is a rise in the AA contents of LD-SD plants. Thus, the content of the shoot apex of the main shoot and branches is high from 134 to 146 days (Fig. 6). This is just about the time when these become reproductive. The AA content reaches a maximum after 156 days. Hence, the AA contents increase with reproductive development and decrease with the formation of seeds.

DISCUSSION

It has been shown by several workers that AA is synthesized in storage tissues and is then translocated to the growing regions (1, 10–12). This fact is supported by the accumulation of AA in the conducting tissues (7). Data presented in this paper indicate that AA is present in all parts of plants throughout the period of growth, lending support to the earlier postulates.

In the present studies, the AA concentration in the shoot apex of *Panicum miliaceum* remains low in the initial stages of growth under all photoperiods. A massive upsurge in AA concentration, however, begins in both the shoot and branch apices when they change from the vegetative to the reproductive condition. This is noted in SD plants only and flowering also occurs only in SD plants. In ND and LD plants, which remain vegetative, the AA concentration also remains low. Moving ND and LD plants to inductive SD conditions results in a synchronous ear emergence and upsurge in AA content. The close correlation of the upsurge in AA concentration with the change in the shoot apex in all cases suggests that AA might have some role to play in the switch-over of the shoot apex to the reproductive state and in the differentiation of floral axis. Earlier flowering in plants caused by exogenous application of AA lends support to these results (2, 6).

These findings are in accord with the earlier ones for wheat (3), barley (8), *Cyperus* (7), and *Setaria* (9). Chinoy (4) has suggested that the formation of AA stimulates the production of nucleic acids and proteins in the shoot apex of a plant, thus increasing the metabolic activity of cells which results in the formation of various organs. In *Panicum miliaceum* also, the AA content increases in the shoot apex suddenly at the time of reproduction. Perhaps this results in the formation of various organizer centers which ultimately form new organs.

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