Effect Of Gibberellic Acid On The Speed And Percentage Of Germination And Vascular Tissue Ontogenesis In Helianthus Annuus L.

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ABSTRACT

Gibberellic acids are among phytal hormones normally involved in breaking seed dormancy, internodal growth, expansion of leaves and increasing resistance against pathogens. Sunflower (Helianthus annuus L.), an oilseed plant, is of great economic importance with pharmaceutical applications. This study used GA3 at concentrations of 25, 50, 75, 100 and 200 mg/l in order to evaluate its effect on the speed and percentage of germination and the vascular ontogeny. Experiments were performed on plants cultured in MS medium as well as those grown in the greenhouse. A significant difference (p<0.05) was observed between the control and 50 mg/l GA3-treated cultured seedlings. 50 mg/l GA3-treated seeds showed the highest speed and percentage of germination. Compared to the control, the highest rate of vascular tissue differentiation was detected in seedlings cultured in MS medium containing 100 mg/l GA3. In general, certain concentrations of GA3, despite lacking the ability to induce fundamental changes in the trend of vascular ontogeny, resulted in a higher rate of differentiation of vascular tissue in young sunflower leaves under both in vitro and in vivo conditions.

Key words: Differentiation, Gibberellic Acid (GA3), Sunflower, Vascular Tissue.

Introduction

Sunflower (Helianthus annuus L.) is an annual plant that grows to a length of up to about 3 meters long. The large and beautiful flowers are capitol type with a diameter of 35cm. It is being grown in most places around the world. The seeds (nuts) are very nutritious with a content of 24% proteins, 47% oil, 20% hydrocarbons, 8% phosphorous and 9% potassium, in addition to vitamins A and B (Khajehpur 2008). The oil content is composed of 65% linoleic acid, some phospholipids and vitamin E. For this reason, sunflower seeds are known to be important in lowering blood triglycerides and in regulating blood cholesterol. The economical and pharmaceutical importance of the plant is mainly due to the valuable and beneficial oil content and other compounds contained within the seeds. These include both saturated and unsaturated fatty acids, folic acid, pantothenic acid, vitamin B6, manganese, iron, selenium and copper [17]. Following extraction of the seed oil, the remaining oil cake can be used for animal feed. Nowadays, through both genetic manipulations and plant improvement programs, sunflower seeds of high quality with higher rates of fertility and more resistance to pathogens have been produced [7]. For the reasons described above on the importance of sunflower, and since germination is a critical stage ensuring reproduction and consequently controlling the dynamics of a population. Plant hormones as regulators of growth have fundamental roles in plant physiology [18,19,21]. This experimental research was set out to investigate in sunflower, the role of GA3 on the speed and percentage of germination as well as the ontogeny of the vascular tissue.

Materials and Methods

Sunflower seeds were provided to us by the Research Institute of Seed and Seedling Improvement of Karaj (Iran). Seeds were first surface sterilized with 30% bleach for 15 min, followed by an 80% alcohol wash for 2 min. Seeds were then washed for three times in sterile distilled water. After the final wash, seeds were plated in petri dishes containing MS medium in a sterile condition. Each dish contained 6 seeds, and each trial was performed in quadruplets. Control lines were grown
in MS medium lacking hormones and vitamins, while experimental samples were grown in the presence of 25, 50, 75, 100 and 200 mg/l of GA3 (added to the culture medium). The number of germinated seeds in each dish was determined, and the percentage and speed of germination was calculated. The speed of germination was calculated by the Maguire (1962) method, according to the following formula:

\[ \text{Speed of germination} = \frac{\text{No. of seeds germinated}}{\text{Days of first count}} + \frac{\text{No. of seeds germinated}}{\text{Days of final count}}. \]

The averages for the percentage of germinated seeds and the speed of germination was calculated using SPSS software ver. 12. Graphs were drawn using SPSS software ver. 15.

To assess the trend of vascular tissue differentiation of 14-day old seedlings, primordial leaves covering the vegetative shoot meristem were removed and stained with chloral lactophenol before examining under a Zeiss stereomicroscope.

Results and Discussions

Germination Data:

Seeds were germinated after 2-days in MS medium (Figure 1), and from day 5 through day 10, seedlings could easily be detected. Significant differences (p<0.05) in speed and percentage of germination could be observed between control and 50 mg/l GA3-treated seedlings. An average speed of germination of 0.78 in the control sample reached a value of 6.01 in 50 mg/l GA3-treated seedlings. The percentage of germination, on the other hand, was 33.33 % and 100% for control and the above-mentioned treated seedlings, respectively. Significant differences (p<0.05) in the average speed of germination could be seen between control seedlings and seedlings treated with 75 mg/l and 100 mg/l of GA3. Differences in the average percentage and speed of germination were also detected between control and 200 mg/l GA3-treated seedlings (Figure 2). In general, the 50 mg/l concentration of GA3 caused the greatest increase in both speed and the percentage of germination. At this concentration of GA3, seedlings were the healthiest with a longer lifespan compared to both control and other GA3-treated seedlings.

![Fig. 1: Comparison of seed germinated in a medium containing 50mg/l GA(A) and the control group(B).](image)

![Fig. 2: Comparisons of of the average percentage (A) and speed (B) of germination of different concentrations of GA with the control group.](image)
Data On The Ontogenesis Of Vascular Tissue:

Formation of the major vein and the minor veins, vascular patterning and the internal branching of the veins were slower in the 75 mg/l GA3-treated seedlings, compared to control and the other GA3-treated groups. The trend in the formation of major vein and the minor veins, formation of the vascular pattern and the internal branching pattern were more intensive in the 100 mg/l GA3-treated seedlings than in the control group (Figures 3 and 4).

Fig. 3. The trend of vascular tissue development in the presence of 50mg/l GA.

Fig. 4. The trend of vascular tissue development in control plants.

The rate of vascular differentiation in seedlings treated with 100 mg/l GA3 was higher than other GA3-treated seedlings, such that the first signs of median vein formation in the initial designing of the leaf (Figure 3-1 E0) could be detected in the 100 mg/l GA3-treated seedlings before appearing in the control group (Figure 4-1).

It is worth noting that GA3 treatment also expedited the overall leaf ontogenesis and caused surface expansion of the young leaf, as well as the appearance of a great number of trichomes at the edges of the leaf (Figures 3 and 4).

At more advanced stages, the formation of minor veins and their branching pattern was faster in GA3-treated seedlings than in the control seedlings of the same age (Figures 3-3 and 4-3).

GA3 treatment resulted in the formation of narrower leaves from this point on. However, the expedition in the formation of the veins due to GA3 treatment continued throughout the later stages of the leaf life (Figures 3-4 and 4-4).

Discussion:

Seed germination is a complex process, initiating with the absorption of water, followed by a short pause, and ending with the synthesis and activation of proteinaceous enzymes. Germination is regulated through a series of interactions of hormonal and environmental factors, and this is possible only when appropriate conditions are met (Aticia et al. 2005).

Gibberellic acid, at different concentrations, has positive effects on germination, and can take on the task of a 5 °C chilling treatment in breaking seed dormancy (Rahmanpour 2006).

In *Eremurus olgae*, treatment with 0.08 M GA3 and 30 mg/l of citric acid results in an optimized seed germination. A 0.08 M GA3 treatment has been shown to increase germination rate in *Eremurus persicus*. At lower concentrations (i.e., 0.001M and 0.005 M), however, GA treatment has negative effects on germination, while treatment with a mixture of GA (0.01 M) and citric acid (50 mg/l) increases germination, in comparison to either one used alone (Rahmanpour 2006).

The positive effect of GA, in particular the 50 mg/l concentration found in this study, on the speed and percentage of germination in sunflower is in line with the results obtained in asphodel (Rahmanpour. 2006), soy bean (Saeedi- Sar 2007), poppy (Fallah 2007) and Cotton (Nasiri 1996).

The acidic nature of GA could be an important factor in making the culture medium more acidic. Therefore, in addition to its role in activating the expression of genes, including alpha-amylase (Lindon 1997), GA may be involved in lowering the pH of the cell wall. This drop in the pH of cell wall may result in the activation of certain cell wall hydrolases. The hydrolysis of the bonds in certain cell wall components would consequently expedite seed germination.

Formation of leaf vascular patterning has astounded the scientists for many centuries. A
complex network of procambial cells emerge from homogeneous subepidermal tissue. Despite the lack of knowledge on the molecular nature of this network, the available data implicate gradually restricted transport routes of the plant hormone auxin in defining sites of procambium formation [16].

Vascular patterning can easily be analyzed during tracheary element differentiation form pre-existing vasculature, however, their identification during the pre-existing vasculature is not an easy task [4].

The characteristics of the structure of a standard dicot leaf such as the venation patterns were initially identified by Hickey [5,6]. The venation patterns of higher dicots form in 3-5 different stages during leaf growth and morphogenesis: 1) The bundle of pre-existing midvein vasculature grows from the lower stem towards leaf primordium in an acropetal fashion (from lower to higher regions of the stem); 2) The bundle of pre-existing secondary vasculature continues to the edge during leaf blade formation; 3) Networks of quaternary and higher order veins, including veins at the free ends, form during leaf growth. These stages, depending on the age of the leaf and the degree of venation, would interfere with growth. These stages of seedling growth, and hence, not only assists in subsequent plant growth, but makes the plant more resistant at times of lower water availability.

Overall, our results indicate that by using certain concentrations of gibberellic acid, in particular the 50 mg/l GA₃, can increase the speed and percentage of germination in sunflower seeds, and assist in a better seedling growth. In addition, using concentrations of up to 100 mg/l GA₃ results in the expedient and optimization of sunflower vascular tissue development, particularly in the early stages of seedling growth, and hence, not only assists in subsequent plant growth, but makes the plant more resistant at times of lower water availability.

References


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