Effect of gibberellic acid on reserve food mobilization of maize (*Zea mays* L. var Arun-2) endosperm during germination

Chandra K Subedi†* and Tribikram Bhattarai‡

† *Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kathmandu, Nepal*
‡ *Amrit Campus, Kathmandu, Nepal*

* *To whom correspondence should be addressed. E-mail: chandraks2000@yahoo.com*

In the first 24 hrs of germination, the dry matter of the growth axis decreased in the control while in 1 mg/l GA_3 solution it increased and in 10 mg/l and 100 mg/l the amount remained the same. Exogenous GA_3 overcomes the dry matter loss in the growth axis during the initial stage and results in an increase in the amount of dry matter. GA_3 application probably mobilized more soluble sugar to the growth axis, which results in an increase in the amount of soluble sugar in the growth axis as compared to caryopsis grown under control. 1 mg/l GA_3 enhanced the amount of soluble sugar and decreased the ether extract. In protein mobilization, 1mg/l and 10mg/l GA_3 solution appeared as effective as other treatments during the period from 48 to 96 hrs after sowing. The germination of seeds correlated directly with the mobilization of endosperm reserve. The seeds treated with 1 mg/l GA_3 solution showed higher mobilization of endosperm reserve, which ultimately showed the higher germination percentage.

Key words: GA_3 mobilization, *Zea mays*, reserve food, protein, soluble sugar, ether extract

Him J Sci **1**(2): 99-102
URL: www.himjsci.com/issue2/ga3

Received: 24 Apr 2003
Accepted after revision: 15 July 2003

Introduction

Germination of seeds involves a rise in general metabolic activity and initiates the formation of a seedling from the embryo. The first step in germination is imbibition of water, which results in swelling of the seed. This water uptake is accompanied by a rapid increase in the respiratory rate of the embryo. Shortly after the absorption of water by the seed, enzyme becomes active. Enzymes such as lipases, proteinases, phosphatases and hydrolases, which help to break down the storage materials, are either activated or synthesized *de novo* (Bewley and Black 1985). The breakdown products are later transported from one part of the seed to another and new materials are also synthesized (Arteca 1997).

The major storage materials in the seed are lipids, proteins and carbohydrates. These storage materials, to a considerable extent, characterize the seeds and they are of course economically the most significant part of the seed. The stored food materials are enzymatically broken down to simpler components and translocated to the embryo, the process known as mobilization, where they provide an energy source for growth.

Most of the physiological activities and growth of plants are regulated by hormones such as gibberellins, auxins and cytokinin. GA_3 was found to enhance root growth, shoot growth, shoot dry weight and accumulation of protein, carotenoids and tissue nitrates in Mangrove species (Kathireasan and Moorthy 1994). The use of exogenous GA_3 also accelerates germination.

Many workers have reported stimulation of endosperm metabolism by the addition of exogenous gibberellic acid. Paleg (1960, 1961) has described the dependence of loss of dry weight, starch hydrolysis and protein release in excised barley endosperm in the presence of added GA_3. Studies with many varieties of barley, wheat and oat have confirmed the generality of this effect (Paleg 1962).

Various studies on maize germination have been carried out by many researchers. Ingle et al. (1964) observed the changes in various chemical components such as sugars, proteins, lipids and nitrogen without exogenously applied GA_3. In the present work various concentrations of exogenous GA_3 (1mg/l, 10 mg/l and 100 mg/l) were applied to test the hormonal effect on germination, dry matter content and mobilization of endosperm reserve.

Materials and methods

Germination of caryopsis

Maize caryopses were obtained from the National Maize Research Programme, Rampur, Chitwan. The maize grains were sun dried. Healthy seeds of uniform size were used for the experiment.

After surface sterilization with 0.1% NaOCl, the caryopses were soaked in distilled water or in varying concentrations of GA_3 solution for 24 hrs and sown in a plastic box (250 mm x 160 mm x 110 mm) containing a double layer of filter paper moistened with distilled water or GA_3 solution. For 120 hrs (5 days), the seedlings were left in the incubator in complete darkness at 28±1°C.

Sample preparation

Twenty seedlings were removed at intervals of 24, 48, 72, 96 and 120 hrs following each treatment. The endosperms and growth axis (parts of seedling besides endosperm were separated by dissection. The dissected endosperms were crushed vigorously with mortar and pestle to form a fine powder that was used to determine the amount of dry matter and reserve food of the endosperm (soluble sugar, protein and ether extract). The growth axes were also dried to determine their dry matter. After drying, the samples were kept in plastic bags and stored at 4°C for further analysis. The dry matter in the sample was determined by using the method described by Bajracharya (1999).

Chemical analysis of endosperm

The amount of water soluble mono- and disaccharides in the sample was determined by anthrone reagent and standard calibration techniques (Welcher 1966) using glucose as the standard.

Total nitrogen was determined by the modified Kjeldhal method (PCARR 1980). The protein content of the sample was determined by multiplying the total amount of nitrogen by 6.25 (cf Bajracharya 1999). The amount of ether extract in each sample was determined by using Soxhlets apparatus, following Paech and Tracey (1955).

The amount of dry matter and endosperm reserve, and the germination percentage of different treatments obtained in this work were the average of three replications.

Results and discussion

Effect of gibberellic acid on seed germination

The percentage of germination increased up to 72 hrs and remained constant afterwards in all treatments and control. Among the various concentrations used in the experiment, 1 mg/l showed the highest percentage of germination (98%) **(Figure 1)**. The stimulatory effect of GA_3 on seed germination has been reported by many researchers (e.g. Lang 1965, Stokes 1965). GA_3 has also been reported to overcome the inhibitory effect induced by abscisic acid on rice germination (Bajracharya and Gupta 1978).

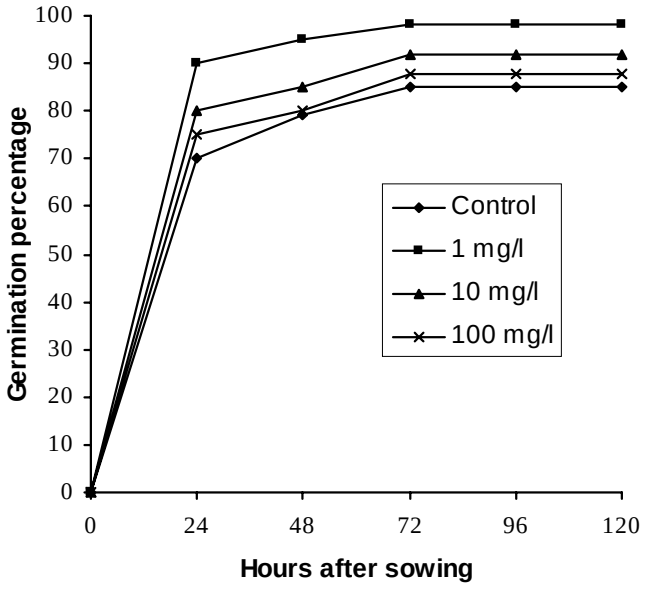

FIGURE 1. Effect of gibberellic acid on seed germination
ANOVA (variance ratio, treatment concentration)
CD = 1.45 at 0.05 level of significance

Effect of exogenous gibberellic acid on dry matter content

For all treatments as well as the control, the dry matter of endosperm decreased gradually with time **(Figure 2)**. The dry matter loss of endosperm was higher in GA_3-treated caryopsis than in the caryopsis grown under control, which indicates that GA_3 enhanced the mobilization of reserve materials from endosperm. GA_3-induced mobilization of reserve materials was also observed by Ingle and Hageman (1965). The greatest loss of endosperm dry matter was observed with 1 mg/l GA_3 treatment. The loss of dry matter decreased as the concentration of GA_3 increased. This shows that GA_3 can enhance the mobilization only up to a certain concentration, above which it appears to be less effective.

In the growth axis, there was loss of dry matter during the initial 24 hrs of germination in caryopsis grown under control **(Figure 3)**. This may have been due to the high rate of respiration in the seedlings after imbibition of water. This respiration was independent of protein synthesis but dependent on substrates stored in the embryonic axis (Abdul-Baki 1969). On the other hand, the dry matter in the growth axis increased during that same initial period with the 1 mg/l GA_3 treatment or remained same with the 10 mg/l and 100 mg/l treatments and decreased under control. This indicates that GA_3 application during germination overcomes the dry matter loss in growth axis during the initial stage and results in an overall increase in the amount of dry matter. After 24 hrs there

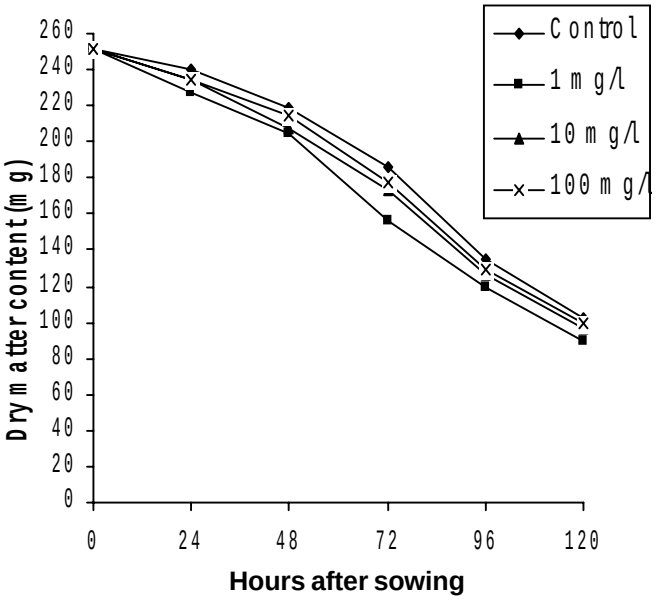

FIGURE 2. Effect of gibberellic acid on dry matter content of endosperm
ANOVA (variance ratio, treatment concentration)
CD = 2.05 at 0.05 level of significance

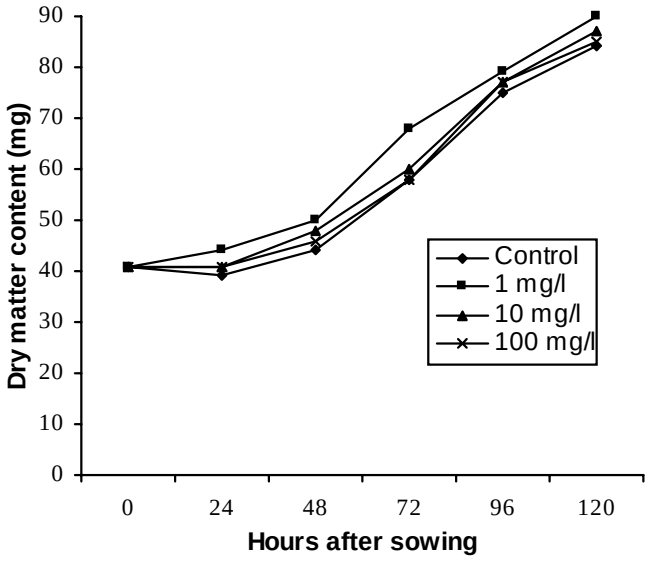

FIGURE 3. Effect of gibberellic acid on dry matter content of growth axis
ANOVA (variance ratio, treatment concentration)
CD = 1.66 at 0.05 level of significance

was a gradual increase in the amount of dry matter in growth axis both in the control and the GA_3-treated plants. This gradual gain in the amount of dry matter was due to the mobilization of food reserves from endosperm. The increase or no change in dry matter of growth axis in GA_3 treated caryopsis in early 24 hrs could be due to the mobilization of reserve food from endosperm to growth axis. The mobilization in the control plants should have started later only after synthesis of endogenous gibberellin, so it showed loss in weight in early 24 hrs as carbohydrate of growth axis was used in its metabolism.

Both in the control and treated plants, the total dry matter gradually decreased during germination **(Figure 4)**. This loss of dry matter is due to the respiratory process. A similar result was also reported by Malhotra (1934) and Ingle et al. (1964).

Effect of GA_3 on mobilization of endosperm reserve

In all treatments and in the control there was a gradual increase in the amount of soluble sugar during germination (**Figure 5)**. GA_3 application accelerated the hydrolysis of starch to soluble sugar by enhancing the hydrolytic enzymes such as α-amylase, β-amylase, maltase and invertase. A similar result was also observed by Salla et al. (1991) in rice. However the soluble sugar concentration was higher in GA_3 treated sample than control in all observations of this work, where endosperm treated with 1 mg/l hormone showed the highest amount of soluble sugar. Endosperm with 100 mg/l GA_3 treatment showed results more or less similar to those of the control. The formation of more soluble sugar in caryopsis treated with 1 mg/l GA_3 as compared to higher concentration treatments suggest that lower concentrations may be more effective in the hydrolysis of starch. The fall in the amount of soluble sugar during the early hrs in the control, followed by an increase after 24 hrs indicates that the conversion of starch to soluble sugar may commence at that point, presumably with the onset of synthesis of endogenous gibberellin. By contrast, caryopsis treated with 1 mg/l GA_3 solution showed a slight increase in the amount of soluble sugar in endosperm in the first day after sowing while at 10mg/l and 100mg/l the amounts remained the same.

As germination progressed the amount of protein stored in the endosperm gradually diminished in the control and in all treated plants (**Figure 6)**. This trend is similar to that observed by Ingle et al. (1964) and Paul and Singh (1981) in lentil seed. The decrease in the amount of protein during germination is explained by the fact that the protein is degraded into soluble nitrogenous compounds through the action of proteolytic enzymes, which in turn are utilized by various parts of the seedling (Mayer and Mayber 1982). The present study indicates that a 1 mg/l GA_3 solution may be more effective in the mobilization of protein (as of sugar) than the higher concentrations tested.

During germination the ether extract was depleted

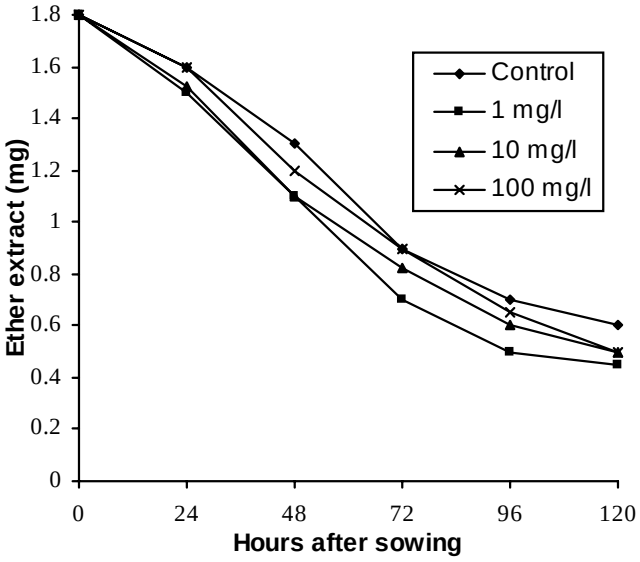

FIGURE 4. Effect of gibberellic acid on dry matter content of seedling as a whole: ANOVA (variance ratio, treatment concentration) CD = 2.14 at 0.05 level of significance

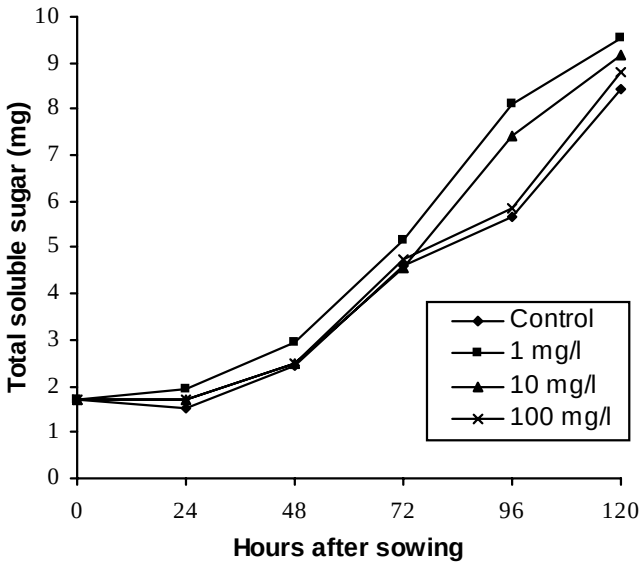

FIGURE 5. Effect of gibberellic acid on solouble sugar mobilization of endosperm: ANOVA (variance ratio, treatment concentration) CD = 0.216 at 0.05 level of significance

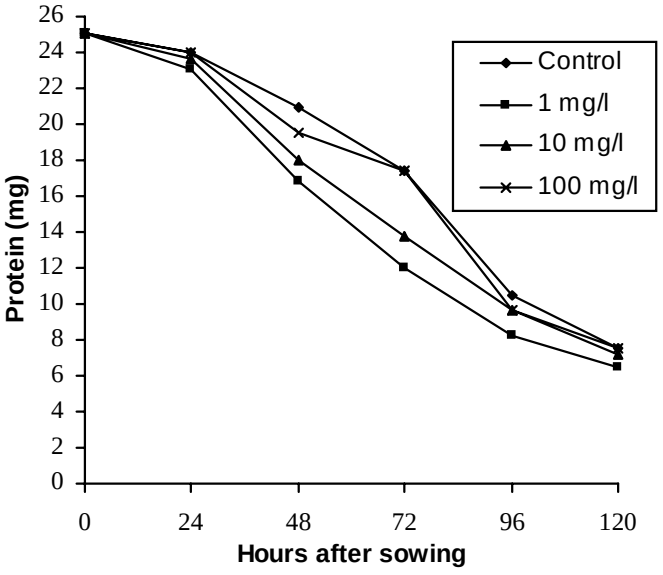

FIGURE 6. Effect of gibberellic acid on protein mobilization of endosperm: ANOVA (variance ratio, treatment concentration) CD = 0.515 at 0.05 level of significance

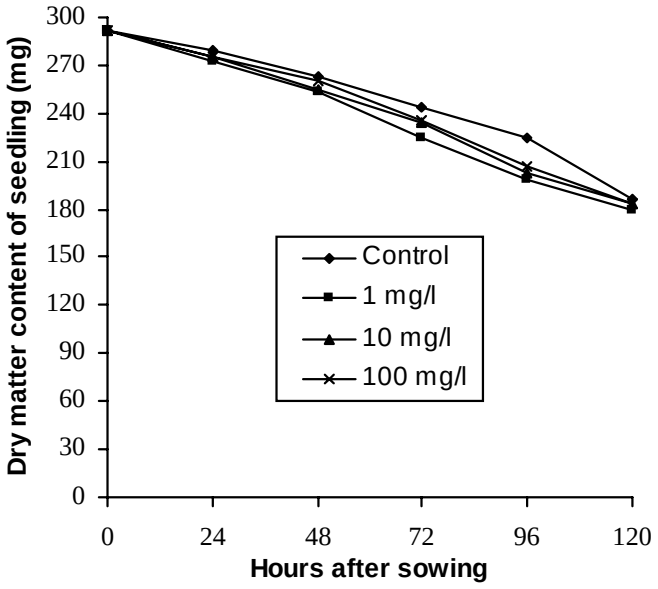

FIGURE 7. Effect of gibberellic acid on ether extract mobilization of endosperm: ANOVA (variance ratio, treatment concentration) CD = 0.02 at 0.05 level of significance

gradually **(Figure 7)**. This depletion of ether extract is possibly due to the conversion of fat into fatty acids and glycerol. Fatty acids are metabolized by glyoxylate cycle to carbohydrate by ß-oxidation. The glycerol is then converted into pyruvic acid or sugars (Stumpf and Bradbeer 1959).

From this investigation, it becomes evident that reserve food mobilization during germination is affected by GA_3 application. GA_3 appears to be effective in dry matter loss also. The loss of increased quantities of dry matter from the endosperm was observed in GA_3-treated caryopsis. This loss was related to the gain of dry matter in the growth axis. But the gain in the amount of dry matter in the growth axis was lower than the loss in the endosperm. This may be due to the consumption of dry matter as a result of respiratory processes in the germinating caryopses (Noggle and Fritz 1991). The increase in the amount of soluble sugar is consistent with the decrease in the amount of protein and fat; their breakdown contributes to the formation of more sugar (Jann and Amen 1977, Stumpf and Bradbeer 1959). Of those concentrations of GA_3 tested, we found 1 mg/l to be most effective in mobilizing the reserve carbohydrates, lipids and proteins. ■

References

Abdul-Baki AA. 1969. Metabolism of barley seed during early hours of germination. *Plant Physiol* **44**: 733-8

Arteca RN. 1997. *Plant growth substances: Principles and application*. New Delhi: CBS Publication. xvi+ 332 p

Bajracharya D and VNP Gupta. 1978. The effect of growth hormones on the germination and dormancy behavior of developing rice (*Oryza sativa* L.) seeds. *J Inst Sci* **I**: 9-14

Bajracharya D. 1999. *Experiments in plant physiology*. New Delhi: Narosa Publishing House. xii+ 186 p

Bewely JD and M Black. 1985. *Seeds: Physiology of development and germination*. New York: Plenum Press

Ingle J and RH Hageman. 1965. Metabolic changes associated with the germination of corn III: Effects of GA_3 on endosperm metabolism. *Plant Physiol* **30**: 672-5

Ingle J, L Beever and RH Hageman. 1964. Metabolic activities associated with the germination of corn: Changes in weight and metabolites and their redistribution in the embryo axis, scutellum and endosperm. *Plant Physiol* **39**: 735-40

Jann RC and RD Amen. 1977. What is germination? In: Khan AA (ed), *Physiology and biochemistry of seed dormancy and germination*. New York: North Holland Company. p 7-28

Kathireasan K and M Moorthy. 1994. Hormone induced physiological response of tropical Mangrove species. *Botanica Mariana* **37**(2): 139-41

Lang A. 1965. Effects of some external and internal conditions on seed germination. In: Ruhland W (ed), *Encyclopedia of plant physiology* **XV**(2): 848-93

Malhotra RC. 1934. Chemistry of corn seed germination. *Cereal Chem* **11**: 105

Mallik CP. 1992. *Plant physiology*. New Delhi: Kalyani Publisher. ix+ 676 p

Mayer AM and AP Mayber. 1982. *The germination of seeds*. New York: Pergamon. ix+ 211 p

Noggle GR and GJ Fritz. 1991. *Introductory plant physiology*. New Delhi: Prentice Hall. xii + 627 p

Paech K and MV Tracey (eds). 1955. *Modern methods of chemical analysis*. Berlin: Springer-Verlag. xv+ 766 p

Paleg LG. 1960. Physiological effects of Gibberellic Acid: On carbohydrate metabolism and amylase activity of barley endosperm. *Plant Physiol* **35**: 293-9

Paleg LG. 1961. Physiological effects of GA_3 - III: Observation on its mode of action on barley. *Plant Physiol* **36**: 829-37

Paleg LG. 1962. Physiological response of GA_3 - V: Endosperm response of barley, wheat and oats. *Plant Physiol* **37**: 798-803

Paul Y and R Singh. 1981. Biochemical changes during germination of lentil seed. *J Res Punjab Agric Univ* **24**(4): 715-9

PCARR. 1980. *Standard method of analysis for soil, plant tissue, water and fertilizer*. Los Banos (Laguna): Farm Resource and Systems Research Division, Phillippine Council for Agriculture and Research. 194 p

Salla M, P Iikka and J Sanna. 1991. Mobilization of storage protein in germinating barley grain. *Luonnon Tutkija* **95**(1/2): 109-13

Stokes P. 1965. Temperature and seed dormancy. In: Ruhland W (ed), *Encyclopedia of plant physiology* **XV**(2): 746-803

Stumpf PK and C Bradbeer. 1959. Fat metabolism in higher plants. *Ann Rev Pl Physiol* **10**: 197-203

Welcher FJ (ed). 1966. *Standard method of chemical analysis*, Vol 3, Part B. New York: D van Nostard Company Inc. xi+ 975-2018 p

Acknowledgements

The authors are thankful to the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, for providing the opportunity to conduct this study and to Nepal Agricultural Research Council (NARC) and Research Center for Applied Science and Technology (RECAST) for the chemical analysis of the endosperm.