식물학회지 제35권 제4호
Korean J. Bot. 35(4) : 333~338

Carbohydrate Metabolism During Germination of Ginkgo (*Ginkgo biloba* L.) Seed

Kim, Myung Ran, Young Soon Kim and Woong Young Soh
(Department of Biology, ChonBuk National University, Chonju)

은행나무 종자의 발아에서 탄수화물 대사

金 明 蘭 · 金 永 順 · 蘇 雄 永
(전북대학교 자연과학대학 생물학과)

ABSTRACT

Ginkgo (*Ginkgo biloba* L.) seeds were analyzed to determine the level of soluble sugars and insoluble starch during germination. Also the activities of the hydrolytic enzymes such as amylase, invertase and phosphatase were compared. As amylase activity was sharply increased, significant decline of starch was observed in the female gametophyte and increase of soluble sugars occurred concurrently. Invertase activity was gradually increased in cotyledon and radicle, while it was very low in dry seeds. In addition, phosphatase activity was variable only in radicle, and acid phosphatase showed higher activity than alkaline phosphatase.

INTRODUCTION

During germination, storage components of seed are hydrolyzed and used as energy source for growth. However, compositional changes associated with germination in gymnosperm seeds (Katsuta, 1961; Ching, 1969) have not been well documented, while intensive work has been conducted with angiosperm seeds (Hsu *et al.*, 1973; Tarrago *et al.*, 1976; Minamikawa, 1979; Monerri *et al.*, 1986).

Seeds of ginkgo are very large and contain large amounts of lipid, protein and carbohydrate as storage components. It also has unusual germination features. The seed holds on to the developing seedling for several months and the cotyledons stay inside the female gametophytic tissue until the seed takes off from the seedling.

The changes in storage materials during germination are the result of the activities of enzymes breaking them down. Generally, enzymes breaking down starch rise in activity fairly rapidly as germination proceeds and it might be possible that these changes are the direct cause of the actual germination in terms of energy source.

Several studies have reported low to zero levels for reducing sugars (i.e., glucose and fructose) in dry pine seeds (Durzan and Chalupa 1968; Baron 1970). Invertase activity has also been reported to be absent in dry seeds of several pine species and eventually developing in association with germination (Hattori and Shiroya, 1951; Hatano and Asakawa, 1964). The same may be said for the development of phosphatase activity in peas (Young *et al.*, 1960). However, it is supposed that these enzyme activities at successive stages of germination remain to be elucidated in especially long lasting seed such as ginkgo seeds.

It is also interesting that part of the embryo takes over these compounds because the large amount of detectable sugars in various parts of the germinating embryo is not only the result of transport from the cotyledons but also the result of breakdown of its own reserved carbohydrate like starch.

In this experiment, we deal with a time-course study of the changes in some related enzyme activities and quantitative shifts in starch and soluble sugars in various parts of germinating ginkgo seeds in relation to break-

down and mobilization of starch reserves. The experimental results offer fundamental information for further studies on the germinating ginkgo seeds so that the nature of long lasting seed with buried cotyledon could be clarified.

MATERIALS AND METHODS

Plant material. Ginkgo (*Ginkgo biloba* L.) seeds were soaked in distilled water for 24 h. The seeds were then germinated in moist sand at 25℃ and harvested at 6 different stages of germination (Fig. 1). Six stages were arbitrarily chosen for study: I) air-dried, II) dehiscent, III) radicle tip emerging, IV) radicle elongating-0.5 cm, V) radicle elongating-1.5 cm and, VI) plumule emerged. The average times of incubation required for attainment of stages II, III, IV, V and VI were 7, 10, 12, 14 and 15 days, respectively. Then, cotyledon, radicle and female gametophyte were cut off and processed for enzyme assay and carbohydrate determination.

Preparation of crude tissue extracts. All cotyledons, female gametophyte and radicle samples were homogenized in a mortar with a pestle in 0.1 M Tris-malate (pH 7.2). The homogenate was centrifuged at 10,000 g for 20 min, and the resulting supernatant was used as an enzyme solution and the insoluble fraction was used for starch determination. For each extract, three assays were carried out and the values were averaged.

Enzyme assays. Amylase (EC 3.2.1.1) was assayed by the method of Bernfeld (1955) using 1% potato starch solution in 0.05 M acetate buffer (pH 5.0) as substratc. Phosphatase activities were assayed spectrophotometrically at 410 nm. For alkaline phosphatase (EC 3.1.3.1.), the reaction mixture contained 50 mM glycine at pH 10, while that for acid phosphatase (EC 3.1.3.2) contained 50 mM sodium-acetate at pH 5. Final reaction mixture contained 50 μl of enzyme solution and 5 mM p-nitrophenyl phosphate (Bonner *et al*, 1988). Invertase (EC 3.2.1.26) activity was assayed in a mixture containing 200 mM sodium-acetate (pH 5.0) and 10 mM sucrose. The activity was determined by measuring glucose released (Doehlert, 1987).

Determination of sugar content. Glucose was determined enzymatically using glucose oxidase and peroxidase (Bergmeyer, 1982). Reducing sugar was measured using dinitrosalicylic acid, according to the method of Miller (1959). Sucrose was hydrolyzed to glucose and fructose by invertase and the glucose produced was measured using glucose oxidase and peroxidase (Waco), according to Tarpley (1986). After amyloglucosidase (Sigma) treatment, starch was measured with the insoluble fraction as glucose content, as described by Schaffer (1985).

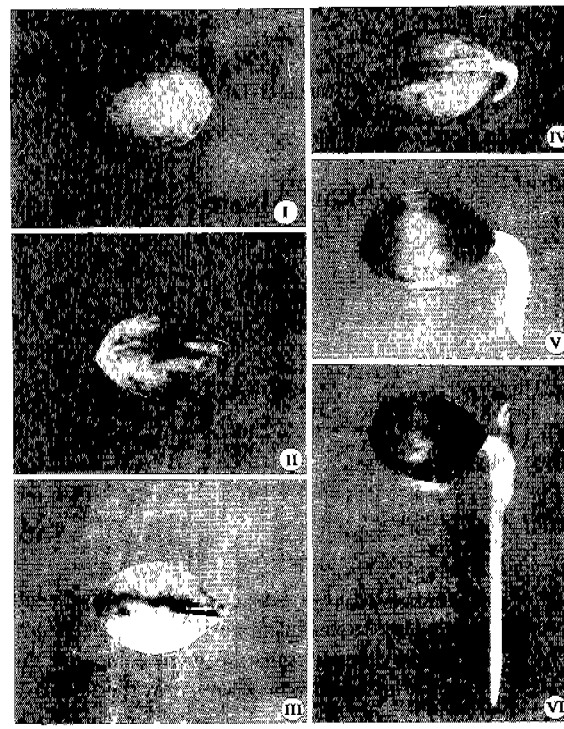

Fig. 1. Photographs of the different stages of germinating ginkgo (*Ginkgo biloba* L.) seeds. I, air-dried; II, dehiscent; III, radicle tip emerging; IV, radicle elongating-0.5 cm; V, radicle elongating-1.5 cm; VI, plumule emerged.

RESULTS

Comparison of changes in hydrolytic enzyme activities and carbohydrate content in female gametophyte, cotyledon and radicle was performed in germinating ginkgo seeds. Each part was removed from germinating seeds at various ages.

Mature ginkgo seeds started to dehisce 7 days after sowing and the radicle became doubled in length while the cotyledon enlarged. Then the radicle elongated rapidly and the shoot emerged 7 days after rupture of the seed coat. But the cotyledons hardly enlarged and stayed inside the female gametophyte.

These samples revealed some trends in the level of carbohydrate content consistent in female gametophyte and cotyledon and radicle as source sink. The level of

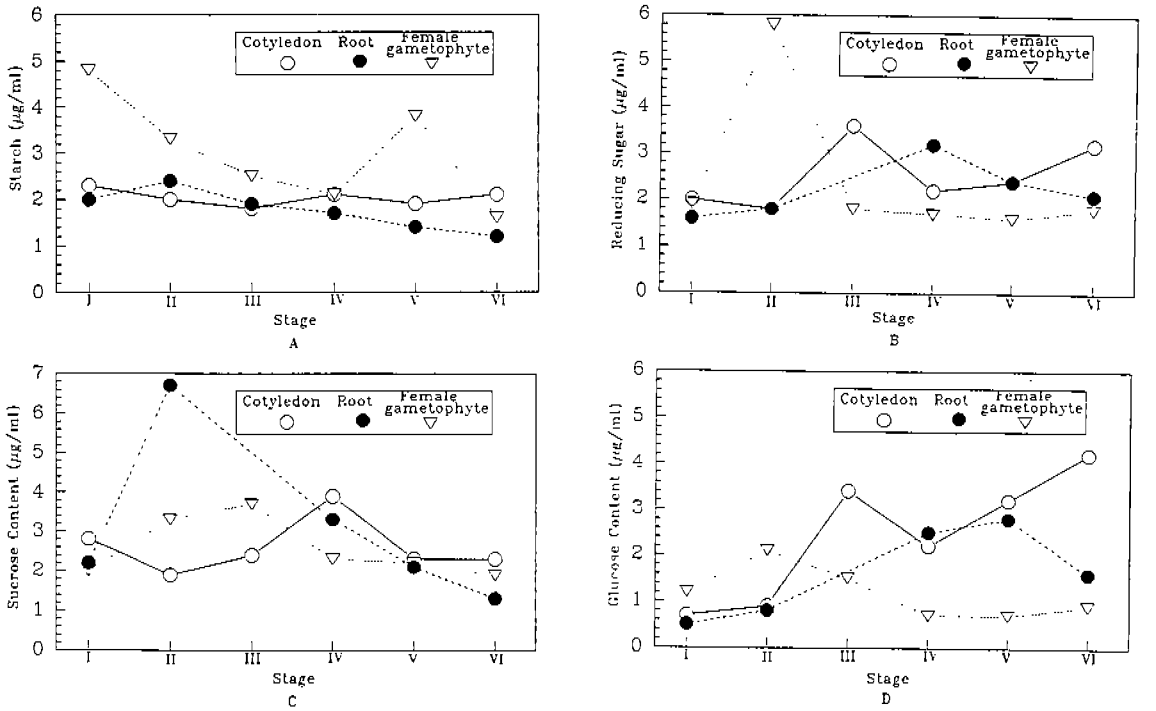

Fig. 2. Changes in amount of various constituents at different stages of ginkgo (*Ginkgo biloba* L.) during germination. A, starch; B, reducing sugar; C, sucrose; D, glucose.

soluble sugar followed increased amylase activity in the first few days.

Amylase activity was sharply increased in the female gametophyte until the radicle tip showed up and started to increase in cotyledon after dehiscent state, then it decreased. In the radicle, it was gradually increased until the shoot emerged (Fig. 3A). The change in glucose tended to be similar to that of amylase activity except for the reduction shown by the female gametophyte (Fig. 2D). The reducing sugar contents also showed a pattern similar to glucose except the female gametophyte in which the sharply increased activity was dropped and maintained thereafter (Fig. 2B).

The pattern of sucrose content showed remarkable difference from other soluble sugars. During the early stage, it showed considerable decline in cotyledon while it increased in the female gametophyte. In the radicle, it was rapidly accumulated in the early stage (Fig. 2C).

A significant decline of starch content was observed in the female gametophyte as amylase activity was sharply increased in both female gametophyte and cotyledon during the early stage of germination (Fig. 3A). However the starch content increased again before the shoot emerged (Fig. 2A).

Invertase, alkaline phosphatase, and acid phosphatase had some tendency in each part of a germinating seed except the invertase activity which was very low in dry seed. The hydrolytic breakdown of sucrose by invertase appeared invariably in the female gametophyte. In contrast, this enzyme avtivity in cotyledon and radicle increased until the shoot emerged (Fig. 3C).

The phosphatase activity showed less substantial changes during germination. Beginning with germination by stage II, the activity declined in all parts and increased during radicle elongation and then was followed by a slow decline. The acid phosphatase had three to four times more activity than alkaline phosphatase (Fig. 3B, D).

DISCUSSION

When the seed germinates, the storage reserves are hydrolyzed and used as energy source and structural elements for growth. Especially, amylolytic enzyme activities should occur in the cotyledons during germination. The role of these enzymes in the breakdown of storage reser-

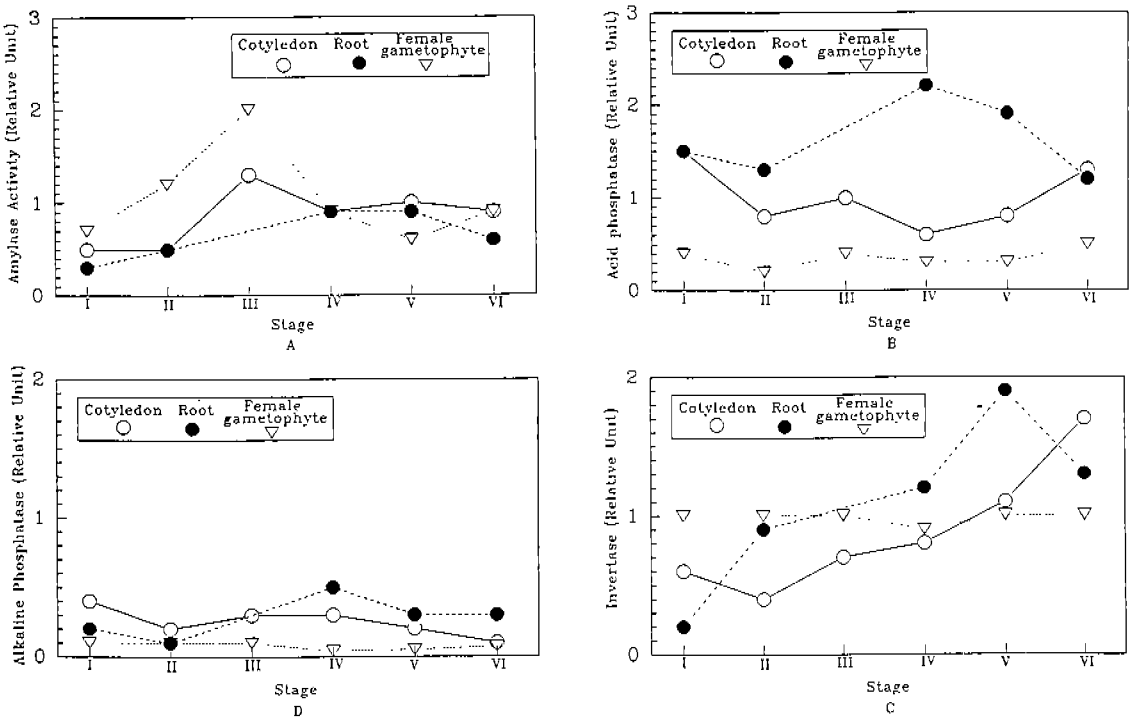

Fig. 3. Changes in the activity of various enzymes at different stages of ginkgo (*Ginkgo biloba* L.) during germination. A, amylase; B, acid phosphatase; C, invertase; D, alkaline phosphatase.

ves has been well reported in lentil seeds (Tarrago *et al.*, 1976), Vigna mungo seeds (Koshiba *et al.*, 1983), *Phaseolus mungo* seeds(Miamikawa, 1979) and pea (Monerri *et al.*, 1986).

Lack of glucose and provision of glucose through the action of amylase and invertase could limit metabolic activity in a dry seed (Hattori and Shiroya, 1951; Hatano and Asakawa, 1964). As soon as conditions requisite for germination were met, several hydrolytic enzyme activities exhibited significant increase (Minamikawa, 1979). It might be possible that these changes are the direct cause of the actual germination in terms of development. As the result, various substances, such as soluble sugars, dissoluted gradually and moved out to other parts of the growing embryo (Meyer and Anderson, 1952).

The change in glucose tended to be similar to that of amylase activity. The reducing sugar contents also showed a similar pattern. However, there were some differences in occurrence of soluble sugars. These differences may result from their metabolic activity in various parts of the seed. In addition, sucrose is often present in dry seeds in small amounts. But sucrose content in radicle showed remarkable difference. It may reflect the increased translocation of sucrose in radicle.

Soluble carbohydrates also undergo fast turn-over during germination. This high availability of sugars may be necessary to meet the high energy requirements to the germinating plant.

Generally, enzymes breaking down starch rose in activity fairly rapidly as germination proceeded (Juliano *et al.*, 1969; Monerri *et al.*, 1986). But the amount of starch in ginkgo decreased once after the germination started and increased again. It seems that the reserved starch was used for radicle elongation and then it accumulated again when it was depleted under a certain level in female gametophyte. This was probably due to the conversion of other metabolites, like proteins and oils, into soluble carbohydrate which may be further involved in gluconeogenesis as reported in caster beans (Yamamoto, 1960). In this way, the accumulated starch in the female gametophyte may be maintained and lasted long as a source sink after germination. Based on the data of starch content and amylase activity in ginkgo seed, it seems that the cotyledon does not have a main influence in the beginning of the germination process. While the female gametophyte takes a major role in storage, the cotyledons

could be expected to be a temporary sink and to transport the dissoluted metabolites. And it might be a possible explanation for cotyledons which are buried in the female gametophytic tissue during germination.

It is interesting that any part of an embryo takes in these dissolved compounds. Apparently, the large amount of detectable sugars in various parts of a germinating embryo is not only a result of transport through the cotyledons but also the result of the breakdown of reserve carbohydrate, such as starch and oligosaccharides. Actually, the vascular system has already developed in a mature embryo (Scheirer and Hillson, 1973; Hong *et al.*, 1985). In ginkgo, the vascular system has completely developed from cotyledon base to hypocotyl-radicle axis in dehiscent seed and it was connected in the growing epicotyl 6 days after dehiscence (Hong and Soh, 1983). In ginkgo seed, hydrolyzed metabolites seemed to be transported to radicle first and then utilized for shoot formation.

적 요

다양한 발달단계에 있는 은행종자의 자성배우체, 자엽 및 유근에서 탄수화물의 함량과 여러 가수분해효소(아밀라제, 인버타제 및 인산가수분해효소)의 활성을 비교하였다. 종자의 초기발아시에 아밀라제의 활성이 크게 증가함에 따라 자성배우체의 전분이 현저한 감소를 보인 반면 수용성 당들은 증가하였다. 인버타제의 활성은 건조한 종자에서는 매우 낮았으며 발아된 자엽과 유근에서 점진적으로 증가하였다. 인산가수분해효소의 활성은 유근이 신장되는 단계에서 변화를 나타내었으며 산성-인산가수분해효소가 알카리성-인산가수분해효소보다 더 높은 활성을 보였다.

REFERENCES

Baron, F.G. 1970. Metabolic patterns in dormant and germinating seeds of sugar pine (*Pinus lambertiana* Dougl.) *Adv. Front. Plant Sci.* **24**: 47-64.

Bergmeyer, H.U., M. Grab and H.E. Walter. 1983. Biochemical reagents for general use. *In*, Methods of Enzymatic Analysis. 3rd ed. Vol. II Verlag Chemie, Weinheim.

Bernfeld, P. 1955. Amylase, α and β. *Methods. Enzymol.* **1**: 149-158.

Bonner, C.A., C. Kenyon and R.A. Jensen. 1988. Pysiological and biochemical characterization of a suspension culture system for sustained exponential growth of *Nicotiana silvestris*. *Physiol. Plant.* **74**: 1-10.

Ching, T.M. 1966. Compositional changes of Douglas fir seeds during germination. *Plant Physiol.* **41**: 1313-13 19.

Doehlert, D.C. and F.C. Felker. 1987. Characterization and distribution of invertase activity in developing maize (*Zea mays*) kernels. *Physiol. Plant.* **7011**: 51-57.

Durzan, D.J. and V. Chalupa. 1968. Free sugars, amino acids and soluble proteins in the embryo and female gametophyte of jack pine as related to climate at the seed source. *Can. J. Bot.* **46**: 417-428.

Hatano, K.I. and S. Asakawa. 1964. Physiological processes in forest tree seeds during maturation, storage, and germination. *Int. Rev. For. Res.* **1**: 279-323.

Hatorri, S. and T. Shiroya. 1951. The sugars in the seeds and seedling of *Pinus thunbergii*. *Arch. Biochem. Biophys.* **34**: 121-134.

Hong, S.S. and W.Y. Soh. 1983. Vascular differentiation in the mature embryo and the seedling of *Ginkgo biloba* L. *Korean J. Bot.* **26**: 207-216.

Hong, S.S., C.D. Lee and W.Y. Soh. 1985. Differentiation of the vascular system in mature embryo and seedling of *Prunus davidiana* FR. *Korean J. Bot.* **28**: 285-296.

Hsu, S.H., H.H. Hardly and T. Hymowitz. 1973. Changes in carbohydrate contents of germinating soybean seeds. *Crop Sci.* **13**: 407-410.

Juliano, B.O.and J.E. Varner. 1969. Enzymic degradation of starch granules in the cotyledons of germinating peas. *Plant Physiol.* **44**: 886-989.

Katsuta, M. 1961. The break-down of reserve protein of pine seed during germination. *J. Jap. Forestry* **43**: 241-244.

Koshiba, T. and T. Minamikawa. 1983. *In vivo* synthesis and turnover of α-amylase in altered and detached cotyledons of *Digna mungo* seeds. *Plant Physiol.* **71**: 173-176.

Meyer, B.S. and D.B. Anderson. 1952. Plant Physiology. 2nd ed. D. van Nostrand Co. Inc., New York.

Minamikawa, T. 1979. Hydrolytic enzyme activities and degradation of storage components in cotyledons of germinating *Phaseolus mungo* seeds. *Bot. Mag. Tokyo* **92**: 1-12.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination reducing sugar. *Anal. Chem.* **31**: 426-428.

Monerri, C.A., Garcia-Luis and J.L. Guardiola. 1986. Sugar and starch changes in pea cotyledons during germination. *Physiol. Plant.* **58**: 618-621.

Schaffer, A.A., E.E. Goldschmidt, R. Goren and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Am. Soc. Hort. Sci.* **110**: 574-578.

Scheirer, D.C. and C.J. Hillson. 1973. The vascular transition region of *Helianthus annus*. I. Bilateral and unilateral patterns of differentiation. *Am. J. Bot.* **60**: 242-246.

Tarpley, L. and J.S. Choinski. 1986. The effect of the embryo axis and exogenous sucrose on lipolysis and glyoxysomal enzyme development in *Ricinus communis* (caster

bean) endosperm and cotyledons. *Physiol. Plant.* **68**: 419-425.

Tarrago, J.F. and G.N. Gregoria. 1976 Starch degradation in the cotyledons of germinating lentils. *Plant Physiol.* **58**: 618-621.

Young, J., R.C. Huang, S. Vanecko., J.D. Marks. and J. E. Varner. 1960. Conditions affecting enzyme synthesis in cotyledons of germinating seeds. *Plant Physiol.* **35**: 288-292.

(Received August 10, 1992)