ORGANIC SUBSTANCE METABOLISM DURING SEED GERMINATION OF *PINUS BUNGEANA*

Shang Jie
Northeast Agricultural University
Zhao Kentian
Northeast Forestry University

ABSTRACT The metabolism of fats, proteins and carbohydrates and change of enzyme of seeds of *pinus bungeana* during the germination were conducted by the methods of gas--liquid chromatography, flow injection, colorimetric analysis of spectrophotometer, to provide theoretical basis for seeds dormancy, germination and storage of seeds of forest tree. The results indicate that (1) carbohydrates were first utilized during germination of seed of *pinus bungeana*; (2) stored substances in seeds began to decompose quickly after radicale broke through seed coats; (3) the activity of enzymes in the seeds does not always coincides with the increase or decrease in quantity of its responsible substance during germination of the seed. Changes in stored substance relate to metabalism of other metabolism of other materials and the use of hydrolysates.

Key Words: Seed, Germination, Metabolism

Pinus bungeana is a special product of China, which is distributed mainly over north and northwest areas. It is one of the rare garden trees in China and was planted in palaces and many gardens long time ago.

Seed germination is the strongest period of life activity in all life periods of a plant and is the basis of a plant forming. During this period, the metabolism of fats, proteins and carbohydrates provides substances and energy for seedling growing. In the past, aforementioned researches were aimed at vegetable and crop seeds, but they were rarely aimed at tree seeds. In this paper, the organic metabolism and the changes of enzymes of seeds of *pinus bungeana* during the germination were resear ched. The main purposes are to provide basis for dormancy, germination and storage of tree seeds and to provide parameters for forestry production.

MATERIALS AND METHODS

Materials and Treating Conditions All seeds of *pinus bungeana* used for this experiment were bought from Beijing in 1986, and then stored in desiccator at 0 ~ 5 ℃ .

The seed germination was accelerated by mixing seeds with sand at high temperature. The detail methods are as following:

First, the seeds were immersed in lukewarm water for 4 days; then, the seeds were mixed with sand (seed sand = 1:3), which were placed into flowerpots; next, the flowerpots were put into temperature cabinet at 25 ℃; finaly, the treated seeds were placed in germinating box to germinate at 19 ℃ . Some treated seeds which the sizes were similar were taken randomly to do all of tests every 3 days during stratification and every 2 days during germination.

Methods The methods of measuring the contents of starch, reducing sugar and proteins were flow injection. GC-9A gas-liqid chromatography was used to measure the content of fatty acids. The activity of starch decomposase and isocitrase was measured by colorimetric analysis of spectrophotometer.

RESULTS AND DISCUSION

Form Change of the Seeds during Germination The seeds of *pinus bungeana* changed from static embryos to seedlings through three main periods seed imbibition, accelerated germination and germination(Tab. 1).

Table 1. The form change of the seeds during germination

Treating time(day)	0 (dry seed)	4 (stratification)	7	13 (put in bed)	15	19
change	original shape	seed inflate	embryo taking embryonic coelom in 2/3	radicale projecting	radicale length is 2.0cm	radicale length is 3.7cm

During above process, the color of cotyledons of the seeds changed gradully from yellow-white to green.

FAT TRANSFORMATION

Changes of Fats and Fat Splitting Enzyme During the seed absorbing water in 0-4th days, the activity of fat splitting emzyme in embryo and endosperm rose rapidly, but fats in both parts rarely decomposed at the same time; in the process of stratification in the 5th-13th days, the content of both in embryo and endosperm decreased slowly as the activity of fat splitting enzyme rose slowly; after radicale broke through seed cover at the 13th day, the activity of fat splitting emzyme in embryo and endosperm rose speedly, and simultaneously, the contents of their fats decreased rapidly in their respective speed. From all above, it is obvious that the changing tend of the activity of fat splitting enzyme in embryo and endosperm of seeds of *pinus bungeana* is generally raised and this results in the content of fats decreasing during the whole process of seed germination (Fig. 1).

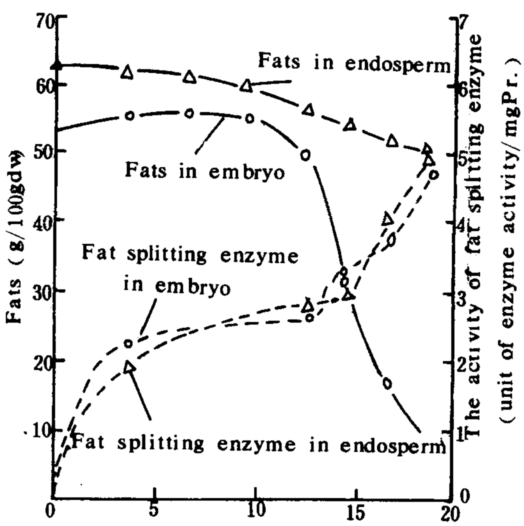


Fig1: Changes of fat contents and activity of fat splitting enzyme during the germination

The greater difference of change between embryo and endosperm is as follows:

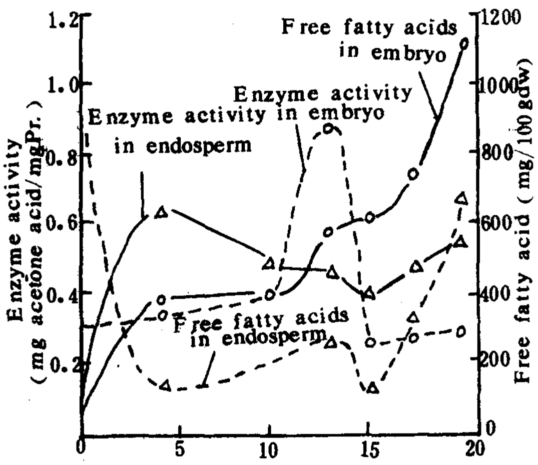
After radicale broke through seed cover at the 13th day, the content of fats in embryo deceased sharply (it decreased 78.39% of total fats in six days of the 13th–19th days.), but in this period, content of fats in endosperm changed within a narrow range(it only decreased 10.14% of total fats.)(Fig. 1). It is thus clear that the seeds of *pinus bungeana* begin to use fats only after radicale broke through seed cover; besides, embryo first takes advantage of its own stored fats.

Change of Fatty Acid Fatty acid, which is main hydrolysate of fats, forms acetyl–CoA by the way of β–oxidation, and then, acetyle–CoA is transformed into succinic acid through acetaldehydic acid circulation. In this paper, the strength of β–oxidation and acetaldehydic acid circulation is expressed respectively by the activity of dehydrase and isocitrase.

The activity of isocitrase and β–oxidation in embryo and endosperm rose obviously in the 0–4th days, and β–oxidation of fatty acid in endosperm reached the highest peak at the 4th day and then it reduced slowly; the activity of its isocitrase fluctuated within a narrow range in the 4th–15th days; the activity of isocitrase and β–oxidation began to rise again from the 15th day, and the activity of isocitrase reached the highest peak at the 17th day, while β–oxidation was rising till the 19th day. The activity of isocitrase and β–oxidation in embryo didn't change obviously during the stratification period, and then they rose speedly from the 13th day (Fig. 2,3).

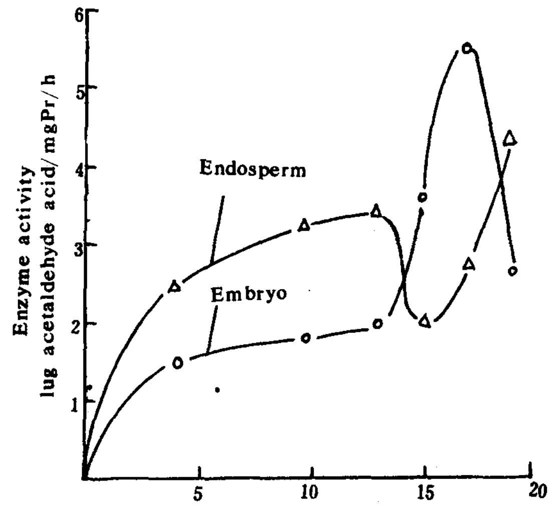
The results of activity change of isocitrase and β–oxidation mentioned above made the content of free fatty acid change (Fig. 2).

Content of free fatty acid in



Days of germination

Fig2. Changes of free fatty acids and β-oxidation during the germination



Days of germination

Fig3. Changes of activity of isocitric acid lyase during the germination

endosperm decreased within a large range in the 0–4th days and at the same time, its content in embryo increased slightly. The reason of this result probably was that free fatty acid transformed from endosperm to embryo during this period, and meanwhile, the activity of isocitrase and β-oxidation in endosperm was greater than them in embryo, which made free fatty acid transform rapidlly, and its product was used for strong respiration of itself and was transported to embryo. During stratification period (5th–13th days), the content of free fatty acid in both parts increased gradully, and its content in embryo reached the highest peak at the 13th day and decreased sharply after radicale broke through seed cover, which provided substantial basis and energy for embryo growing. Content of free fatty acid in endosperm decreased gradually in the 13th–15th days and reached the lowest point(Fig. 2). At the same time, fat content in endosperm also decreased gradually (Fig. 1), and activity of isocitrase and β- oxidation reduced too. Thus, it is thought that this result is because free fatty acid was transported to embryo to ensure its speedy growing. From 15th day, though activity of both isocitrase and β- oxidation rose again, fatty acid produced by hydrolysis didn't transform wholly, and meanwile, fatty acid in embryo is enough for its own need. Thus, during this period, fatty acid in endosperm accumulated (Fig. 2).

PROTEIN TRANSFORMATION

During germination period, total changing tend of content of protein (it is 20.35% of entire seed weight) of seed is: the content in embryo increased gradually and that in endosperm decreased gradually. It is clear that the proteins in endosperm is provided to embryo for its growing. But, total changing range of protein is narrow (increment in embryo is 4.5 g/100g.dw, reduce in endosperm is 2.9g/100g. dw) because stored proteins which was decomposed was used again for forming new structure protein and enzyme protein(Fig. 4).

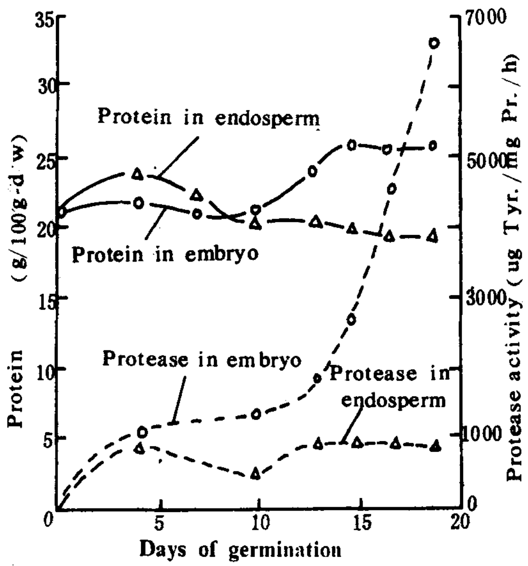


Fig4. Changes of protein contents and protease activity during germination

During the whole germination period, protease activity in embryo first rose obviously and grow up sharply during speedy growing of embryo. From begining to end, protease activity in endosperm didn't change obviously (Fig. 4). It is clear that protease activity of seeds of *pinus bungeana* is irrelated to content change of proteins.

CARBOHYDRATE TRANSFORMATION

Change of Starch And Amylase Starch content of seeds in *pinus bungeana* is 7.83%. From the begining of seed absorbing water, starch content both in embryo and endosperm decreased gradually, which was related to amylase activity rising in two parts, after radicale broke through seed cover, though amylase activity in embryo grew up rapidly, its starch content increased too. It is thus obvious that starch content at right time is irrelated to amylase activity, and starch increasing is mainly because photosynthesis produced sugar during the late period of germination; besides, fat decomposation provided a deal of sugar. At this moment, providation was larger than needs, and it stored by the way of synlysis starch. During this period starch content in endosperm changed within a narrow range (Fig. 5).

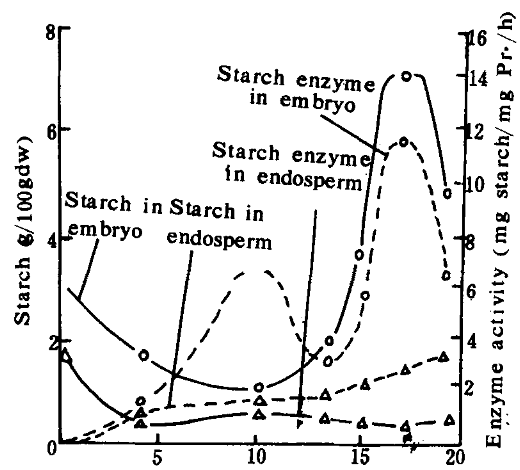


Fig5. Changes of starch contents and activity of starch enzyme during the gemination

Change of Reducing Sugar Reducing sugar is main structrue substance of embryo transforming to seedling. Reducing sugar content of seeds of *pinus bungeana*, which is very low (it is only 1.91% of total seed.), does not change until embryo root broke through seed cover, the contents in embryo and endosperm rose rapidly because hydrolyzatrs of starch, fat and protein are more than needs of seed respiration and growing and they transform to reducing sugar. As for reducing sugar content rising in endosperm later for 5 days, it is probably because reducing sugar transforms from endosperm to embryo.

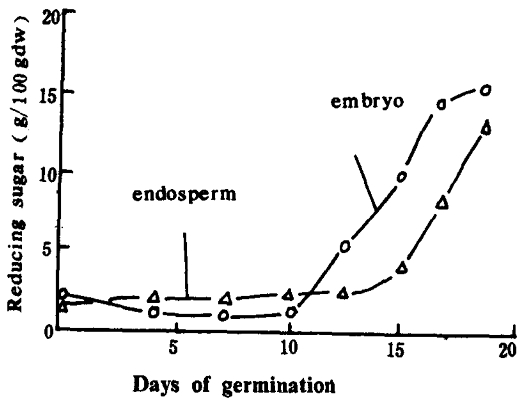


Fig6. Changes of reducing sugar contents during the germination

CONCLUSION

1. Carbohydrates were first utilized during germination of seed of *pinus bungeana*;

2. Stored substances in seeds began to de compose quickly after radicale broke through seed cover;

3. The activity of enzymes in the seeds does not always germination of the seed. Change in stored substance relate to metabolism of other materials and the use of hydrolysates.

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