MEDWELL PUBLISHING
Plant Sciences Research 1 (4): 74-77, 2008
ISSN: 1995-476X

Changes of Fresh Weight, Contents of Dry Mass, Soluble Carbohydrates and Soluble Proteins During Development of Loquat (*Eriobotrya japonica* Lindl.) Anther-Derived Embryos

[1,2]Hongmei Qin, [1]Yongqing Wang, [1]Runzhi Gu and [3]Guanglun Ruan
[1]College of Forestry and Horticulture, Sichuan Agricultural University, Ya'an 625014, Sichuan, China
[2]College of Life Science, China West Normal University, Nanchong 637002, Sichuan, China
[3]Fruit Administration of Fuling Distribution, Chongqing 408000, China

Abstract: Histological studies revealed that the anther-derived embryos of loquat developed through the typical globular, heart, torpedo and cotyledon stages. The changes of fresh weight, contents of dry mass, soluble carbohydrates and soluble proteins were studied during the development of anther-derived embryos of Loquat. The fresh weight increased with the anther-derived embryos developing. With the largest increment from the torpedo stage to cotyledon stage, the dry mass trended to increase at a low level, which was the lowest at the heart stage. In the embryogenic callus formed from anther-derived embryo, the contents of soluble carbohydrate were the most. The globular anther-derived embryos have a higher level of contents of soluble carbohydrates following in the embryogenic callus. But the embryos at globular, heart, torpedo and cotyledon stages kept a low-level contents of soluble carbohydrates and changed with a small extent. The contents of soluble proteins were high in embryogenic callus formed from anther-derived embryo and globular embryos. But it fall with different level at all next stages.

Key words: Fresh weight, dry mass, soluble carbohydrate, soluble protein, anther-derived embryos

INTRODUCTION

As an important economic fruit crop, loquat (*Eriobotrya japonica* L.) is widely cultivated between 20° and 35° latitude including China, Japan, India, Pakistan, Madagascar, Reunion Island, Mauritius Island, the Mediterranean countries (Spain, Turkey, Italy, Greece, Israel), the United States (mainly California and Florida), Brazil, Venezuela and Australia (Badenes *et al*., 2000; Vilanova *et al*., 2001). It has been reported the establishment of plant regeneration system and haploid production through embryogenesis from anther culture of loquat (Li *et al*., 2008).

Plant regeneration by somatic embryogenesis or pollen embryogenesis has been achieved on many species. It offered, favorable experimentation system for plant cell's differentiation, development, totipotency expression, crop variety improvement, screening of Mutant, etc. Despite the fact that Somatic embryos are genetically identical to the donor plant and are morphologically similar to zygotic embryos in early stages of development (Dodeman *et al*., 1997). Their physiological and biochemical dynamic trend are similar as well as have distinct difference. The dry mass accumulation, protein yield and content of carbohydrate of mature somatic embryos are significantly lower than corresponding zygotic embryos. It is possibly, the main reason of low maturation, germination and conversion rates (Chanprame *et al*., 1998; Alemanno *et al*., 1997). The research of changes of fresh weight, contents of dry mass, soluble carbohydrate and soluble protein during development of loquat anther-derived embryos will provide reference to study the action mechanism of germination.

MATERIALS AND METHODS

Plant materials: Loquat anther-derived embryos got multiplications on solid MS medium containing 0.05 mg L^{-1} ZT, 0.02 mg L^{-1} NAA and IBA and 30g L^{-1} sugar. The newly formed embryos were attached loosely to the mother calluses with a short suspensor-like structure at the basal end and they could easily be detached from parent calluses like the primary embryos. Selected the embryos at the stages of typical globular, heart, torpedo and cotyledon, also the mature cotyledon stage and the embryogenic callus formed from anther-derived embryo as the research objections.

Corresponding Author: Yongqing Wang, College of Forestry and Horticulture, Sichuan Agricultural University, Ya'an 625014, Sichuan, China

Methods: Pick out a certain number of globular, heart, torpedo and cotyledon embryos, respectively under dissecting microscope and measure their fresh weight. And then calculate their respective average single embryo's fresh weight.

Pict out a certain number of the embryogenic callus formed from anther-derived embryo, globular, heart, torpedo, cotyledon and matured cotyledon embryos, respectively under dissecting microscope and measure their fresh weigh. And then desiccate with drying by reagents and measure their dry weigh.

The determination method of soluble carbohydrate content in Loquat anther-derived embryos was by using Antrone Chromametry method.

The determination method of protein content in Loquat anther-derived embryos was by using the dying method with Coomassie brillant blue G-250.

RESULTS

Change of fresh weight: In all developmental stages, the fresh weight of single loquat anther-derived embryo was light, though the fresh weight of single embryo increased gradually in different degree at different developmental stage (Table 1). The increment of fresh weight of single embryo was different at different stage (Fig. 1). It may be mean that, fresh weight of the embryos in all developmental stages changed at different levels. From globular stage to heart stage, the increment of fresh weight of single embryo was the least. And from torpedo stage to cotyledon stage, the increment of fresh weight of single embryo was the most.

In all developmental stages, the water contents of anther-derived embryos were high and the dry mass contents were low. At the heart stage, the dry mass percentage of embryo was the minimum (7.425%). And at the mature cotyledon stage, the dry mass percentage was the maximum (16.691%). In embryogenic callus formed from anther-derived embryo, typical globular, heart, torpedo and cotyledon stage, the dry mass percentage of embryos changing with comparatively low degrees. But at the mature cotyledon stage, the dry mass percentage was markedly higher than that of others (Fig. 2). It probably means that the dry mass accumulated more during the cotyledon embryos' maturation process.

Change of soluble carbohydrate contents: The soluble carbohydrate content in embryogenic callus formed from anther-derived embryo was higher than all others stages of embryos. At the stage of globular stage, the soluble carbohydrate content of embryos declined with a large extent but still higher than that of next stages. At the stages of heart, torpedo, cotyledon and the soluble carbohydrate content kept low and changed with small levels. But in the mature cotyledon embryos, the soluble carbohydrates content enhanced a little, which still was lower than that of globular stage (Fig. 3).

In the embryogenic callus formed from anther-derived embryo and globular embryos, the protein contents were high. But at the next stages, the protein contents descended gradually (Fig. 4). In the heart embryos, the protein contents were 3.45% and it reached 1.78% in the mature cotyledon embryos. The protein contents in

Table 1: The fresh weight of embryos at different stages

Stages	Number of embryos	Total fresh weight (g)	Single fresh weight (mg)
Globular stage	190	0.144	0.758
Heart stage	192	0.177	0.921
Torpedo stage	160	0.230	1.438
Cotyledon stage	159	0.357	2.245

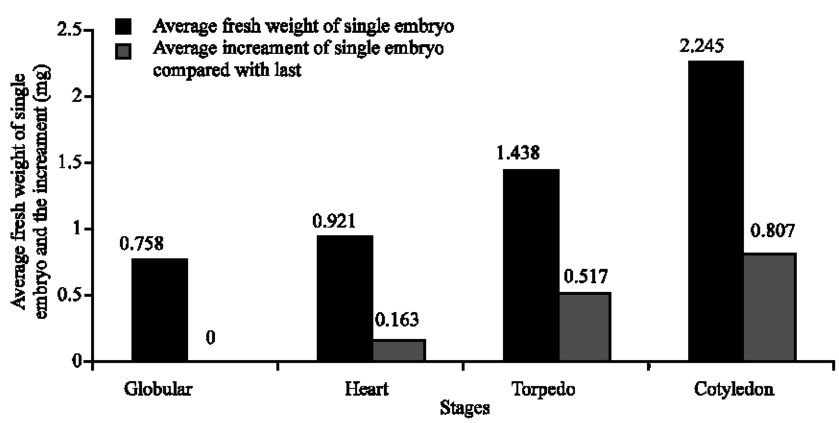

Fig. 1: The change of fresh weight of single embryo at different stages change of dry mass contents

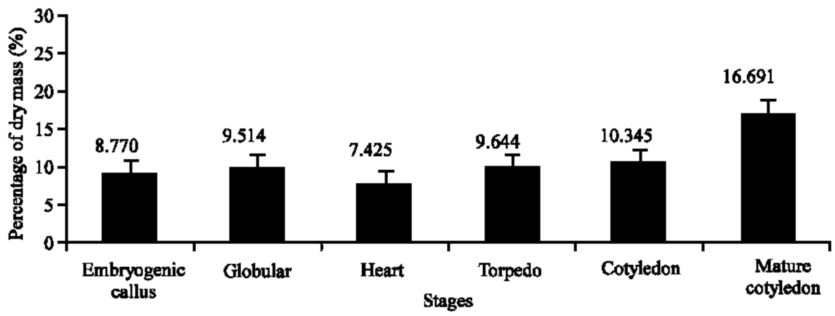

Fig. 2: Percentage of dry mass in embryos at different developmental stages

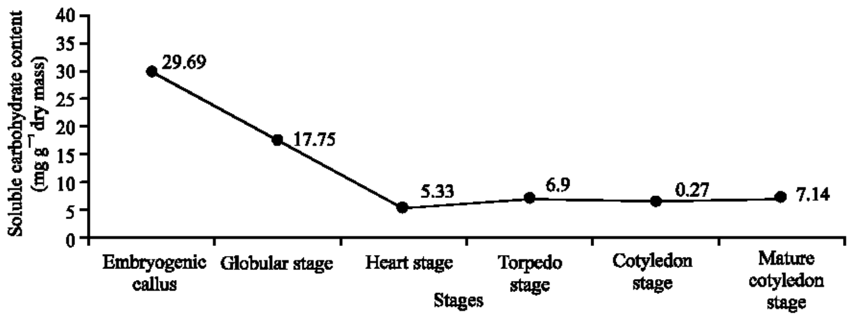

Fig. 3: The content of soluble carbohydrate in embryos at different developmental stages changes of soluble protein contents

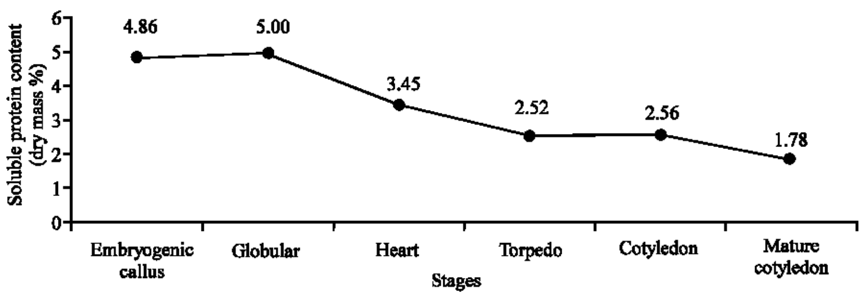

Fig. 4: The content of soluble protein in embryos at different developmental stages

torpedo embryos and cotyledon embryos just changed a little. As a whole the soluble protein contents declined with the embryos developing.

DISCUSSION

The volume of loquat anther-derive embryo was increased gradually during development and so did its' fresh weight. But still the anther-derive embryo was very small and light, which was different from the homologous zygote embryo. This phenomena also been observed in other plants (Lu *et al.*, 1990).

The loquat anther-derive embryo had high water content in all developmental stages, which also result in low dry mass percentage. The change trend of dry mass content suggested that the dry mass of anther-derive embryo accumulated with a low level. The minimum dry mass percentage was the heart shape embryo. One of the possible reasons was the lower biochemistry reactivity at this stage.

The compositions of soluble carbohydrate are diversiform. The high soluble carbohydrate content in embryogenic callus formed from anther-derived embryo maybe partially relate with the sugar content in culture medium. As a whole the soluble carbohydrate content declined gradually at embryogenic callus, globular and heart stages shown in Fig. 5. But it had a small level increased in the next stages.

The composing of protein established a molecular foundation to somatic embryogenesis and development. At globular stage, the protein contents accumulated more, which provided corporeal foundation for embryo's further

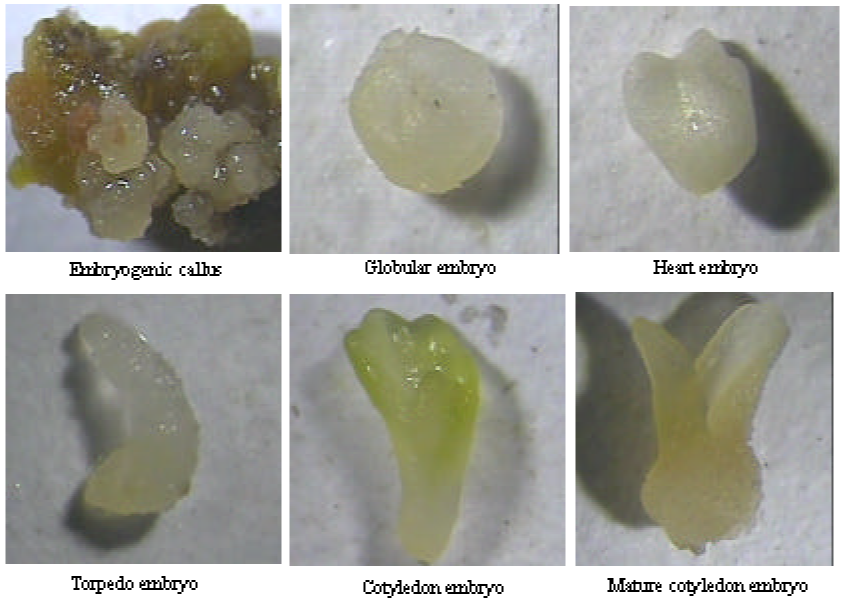

Fig. 5: Soluble carbohydrate context declined gradully at embryogenic callus, globular and heart stages

development. In this research, protein contents declined in the mass with the anther-derive embryo's development. The same change trend of protein content also be observed in other somatic embryos (Krochko *et al*., 1992, 1994).

CONCLUSION

The fresh weight of single loquat anther-derived embryo increase during developing process. In all developmental stages, the dry mass accumulate in a low level. The soluble carbohydrate contents depress obviously at early stage and increase slowly at next stages. As a whole, the soluble protein contents decline with the embryos developing. All the changes reveal physiological and biochemical dynamic trend in the loquat anther-derived embryos during development.

REFERENCES

Alemanno, L., M. Berthouly and N. Michaux-Ferriere, 1997. A comparison between *Theobroma cacao* L. zygotic embryogensis and somatic embryogenesis from floral explants. InvitroCell. Dev.Biol.-Plant., 33: 163-172. Doi: 10.1007/s11627-997-0016-8.

Badenes, M.L., J. Mrti´nez-Calvo and G. Lla´cer, 2000. Analysis of a gerplasm collection of loquat (*Eriobotrya japonica* L.). Euphytica., 114: 187-194. Doi: 10.1023/A:1003950215426.

Chanprame, S., T.M. Kuo and J.M. Widholm, 1998. Soluble carbohydrate content of soybean [Glycine max (L) Merr.] somatic and zygotic embryos during develoment. In vitro. Cell. Dev. Biol. Plant., 34:64-68. Doi: 10.1007/BF02823125.

Dodeman, V.L., G. Ducreux and M. Kreis, 1997. Zygotic embryogenesis versus somatic embryogenesis J. Exp. Bot., 48: 1493-1509. Doi: 10.1093/jxb/48.8.

Krochko, J.E., D.J. Bantroch, J.S. Greenwood and J. Derek-Bewley, 1994. Seed storage proteins in developing somatee embryos of alfalfa: Defects in accumnlation compared to zygotic embryos. J. Exp. Bot., 45: 699-708. Doi: 10.1093/jxb/45.6.

Krochko, J.E., S.K. Pramanik and J. Derek-Bewley, 1992. Contrasting storage protein synthesis and messenger RNA accumulation during development of zygotic and somatic embryos of alfalfa (*Medicago sativa L.*). Plant Physiol., 99: 46-53. URL: http://www.plantphysiol.org/cgi/reprint/99/1/46.

Li, J.Q., Y.Q. Wang, L.H. Lin, L.J. Zhou, N. Luo, Q.X. Deng, C.X. Hou and Y. Qiu, 2008. Embryogenesis and plant regeneration from anther culture in loquat (*Eriobotrya japonica* L.). Scientia Hort., 329-336. doi:10.1016/j.scienta.2007.10.007.

Lu, T.G., Y.S. Wang and G.C. Zheng, 1990. Changes of contents and synthetic activities of DNA, RNA and protein during development of Maize pollen embryos. Acta Genetica Sinica., 17 (6): 449-454.

Vilanova, S., M.L. Badenes, J. Martines-Calvo and G. Lla, 2001. Analysis of germplasm (*Eriobotrya japonica* Lindl.) by RAPD molecular markers. Euphytica., 121: 25-29. Doi: 10.1023/A:1012051207948.