韓作誌(Korean J. Crop Sci.) 33(3) : 248~253(1988)

Studies on the Viability of Cultured Anther in Rice Anther Culture

I. Changes of Respiratory Activity by Genotype and Cold-pretreatment

Seung Yeob Lee*, Seon Yong Lee* and Jang Soo Choi*

벼 培養葯에서 葯의 活力 硏究

I. 品種 및 低溫 前處理에 따른 呼吸活性의 變化

李承燁* · 李善龍* · 崔章洙*

ABSTRACT

The longer pollen stage grew to flowering stage, the higher anther respiratory rate *in vivo* became, and it was rapidly increased just before flowering. The anther respiratory rate *in vitro* showed the first and second peak points after 3-7 days and 9-11 days in culture, respectively, and fastest and highest in Daecheongbyeo with high sporophytic potentiality. It was lower in cold-pretreatment than non-treatment at the early days, but higher from 15 days after culture.

The frequency of browning anthers was promoted by cold-pretreatment. The respiratory rate was not different between uncolored and browned anthers at 12 days, but it was higher in browned anthers after 24 days in culture.

INTRODUCTION

The frequency of callus formation in anther culture was greatly different by genotype, physiological status of donor plant, culture condition, pretreatment and so on. The induction of androgenic haploids in rice showed especially a striking *contrast* by *genotype*.[2,14] Recently, the physiological status of donor plant has been recognized as an impotant factor in haploid induction.[12,18] Also, the effect of cold-pretreatment on anther response has been reported to be the most significant.[3,6,13] The growth conditions during the meiotic division stage in rice plant were very impotant in anther culture. Low temperature during meiotic division stage increased the production of abnormal pollen grains which had been described as pollen dimorphism in anther culture.[5,7] An anther activity has been mainly indicated by the measurement of ATP content and respiratory rate.[17,19] It has been known that the anther respiratory activity was closely related to chilling resistance. This was concerned with mitocondria activity in anther wall and pollen grains. Leaver[10] might draw parallels between mitochondrial condensation and embryogenic potential on the one hand, and mitochondrial attenuation and male sterillity on the other, in the cytoplasm distribution of pollen grains. Also, the condensation of mitochondria may help pollen grains turn towards the sporo-

* 湖南作物試驗場(Honam Crops Experiment Station, Iri 570-080, Korea) 〈'88. 6. 22. 接受〉

phytic pathway.[15]

Therefore, we think that the activity of cultured anther was closely related to callus induction, At first, present work was corried out to investigate the change of respiratory rate by genotype and cold-pretreatment in cultured anthers.

MATERIALS AND METHODS

Anther culture

The materials used in this experiment were Indica-Japonica cross (cv. Milyang 23) and Japonica (cv. Daecheongbyeo, Chiagbyeo) rice. When the auricle of the flag leaf was 4-5cm above the auricle of the second leaf, the panicles of late-uninucleate microspore stage were collected for inoculation. The cold-pretreatment of panicles was treated for 10 days at 10℃. Fifteen intact anthers from the spiklets in middle part of the anther donor panicles were transferred to the test tube (1.8 x 15cm) which contained 7 ml solidified N_6 medium.[4] The culture condition was maintained at 27±1℃ under dark condition throughout the course of experiment.

Measurement of respiratory rate

The anthers of five stages from early microspore stage to just before flowering stage namely, early-and middle-uninucleate microspore stage, late-uninucleate and early-binucleate pollen stage, middle-binucleate pollen stage, late-binucleate pollen stage and just before flowering stage, were collected from three specific spikelets,[9] and 60 anthers were used for respiration measurement before culture. Also, one hundred sampled anthers were collected at interval of 2 or 3 days from one day after culture and used for respiration measurement.

After the collected anthers were enmeshed in the nylon net, they were soaked in distilled water and infiltrated under reduced pressure. The anthers were transferred into a measurement vessel with 3 ml of O_2 saturated buffer solution containing 50 mM HEPES and 0.5 mM $CaSO_4$ at pH 7.2, and preincubated at 27℃ for 5 min. The O_2 uptake of the anthers was measured by an oxygen electrode (Rank Brothers Engineering, England). The rate of O_2 uptake was measured for about 10 min. The respiratory rate was presented as n mol of O_2 uptake per hour per anther.

Brownish frequency and respiratory rate in cultured anther

For comparison of anther activity, the brownish frequency was investigated at intervals of 2-3 days after culture in the cultured anthers of Milyang 23 with or without the cold-treatment. Also, the respiratory activity between uncolored and browned anther was compared at 12 and 24 days after culture, and 100 anthers were used for respiration measurment.

RESULTS

The respiratory activity *in vivo* was investigated in anthers of five stages from early microscopore stage to just before flowering stage (Fig. 1). The

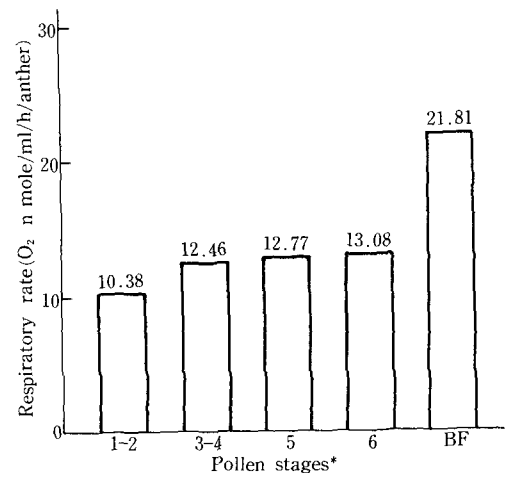

Fig. 1. Changes of respiratory rate by different stages in Milyang 23.

* Stage 1-2 : Early-and middle uninucleate microspore stage
Stage 3-4 : Late-uninucleate and early-binucleate pollen stage
Stage 5 : Middle-binucleate pollen stage
Stage 6 : Late-binucleate pollen stage
BF : Just before flowering

longer pollen stage grew to flowering stage, the higher respiratory rate became. Then it was rapidly increased just before flowering and showed about 21.81 O_2 n mol/ml/h/anther.

The changes of respiratory rate according to genotype and cold-pretreatment were investigated in the cultured anthers (Fig. 2 and Fig. 3). The respiratory rate of cultivars was showed the highest activity in Daecheongbyeo with high sporophytic potentiality. Then the change of respiratory rate *in vitro* showed two peak points. The first and second peak points in Deacheongbyeo were reached at 3 and 9 days after culture. The first peaks in Chiagbyeo and Milyang 23 were reached at 3 and 7 days after culture respectively, but the second peak point was not clean-cut.

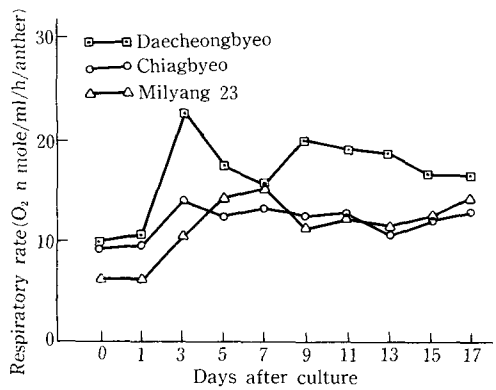

Fig. 2. Changes of respiratory rate by genotype in cultured anther.

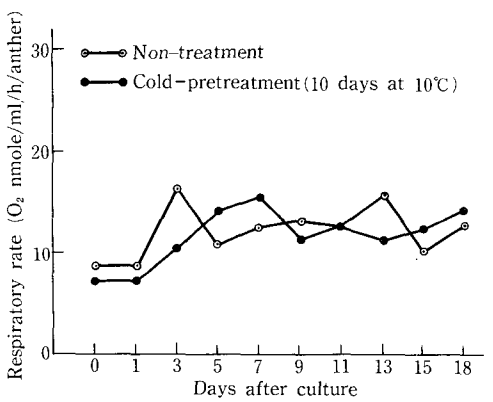

Fig. 3. Changes of respiratory rate by cold-pretreatment in cultured anthers of Milyang 23.

The respiratory rate by cold-pretreatment in Milyang 23 was not the uniform tendency between cold-pretreatment and non-treatment.

Also, the change of anther wall tissue was obserbed at intervals of 2 or 3 days (Fig. 4). The browning of anther was faster in cold-treatment than in non-treatment. The frequency of browned anther reached to over 50% in cold-pretreatment after 9 days, whereas in non-treatment after 17 days in culture.

The respiratory rate was measured to compare the anther viability between browned anthers and

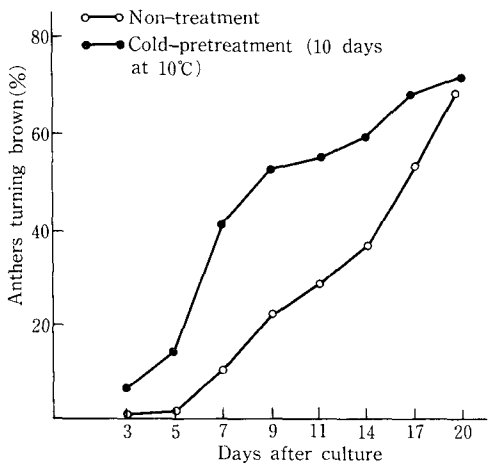

Fig. 4. Effect of cold pretreatment on the frequency of browned anther after culture (cv. Milyang 23)

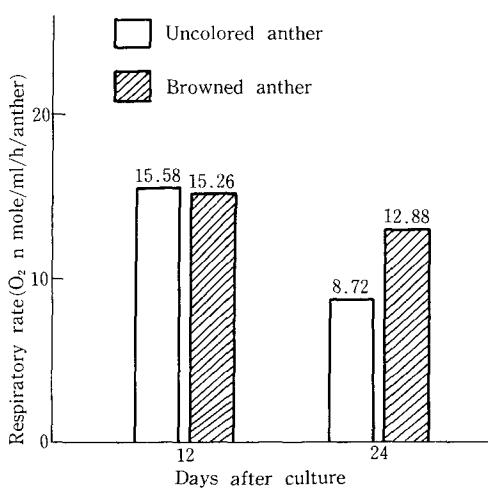

Fig. 5. Comparison of respiratory rate between uncolored and browned anther after culture(cv. Milyang 23)

uncolored ones at 12 and 24 days (Fig. 5). It was not different at 12 days, but it was much higher in browned anthers than in uncolored ones 24 days after culture.

DISSCUSSION

It seemed that the change of respiratory rate was more affected by anther wall tissue than microspores at early date, but it was more closely related to the development of microspores at latter. Two peak points in Daecheongbyeo and Chiagbyeo (Jap.) were faster and higher than those in Milyang 23 (Ind./Jap.). It was inferred that the result had some connection with sporophytic potentiality. The first peak point formed at 3-7 days. The cause seemed not only that most of anther wall tissue and pollen grains were viable, but that normal pollen grains actively made cytoplasm synthesis through the first pollen mitosis at this date. Then, respiratory rate was lower because of the deterioration of anther wall tissue and normal pollen grains. The second peak point revealed at about 9-15 days. It seemed to be caused by the activation of laggard normal pollen grains and sporophytic pollen grains. This suggestion agreed to the results investigated by Yang and Zhou.[21] They observed that 2-celled and multicellular pollen grains were rapidly increased after 3-4 and 10-12 days in culture, respectively. Also, the change of respiratory rate by cold-pretreatment in Milyang 23 was lower than non-treatment at early days, whereas it was more increased from 15 days after culture. This phenomenon was related to anther activity. The low or high temperature and shading treatments decreased ATP content in anther.[17] And Toriyama and Hinata[19] reported that anther sterility under low temperature was closely correlated with the depression of respiratory activity, and that active respiration is probably indispensible to the development of fertile anthers.

The present experiment inferred that the pollen grains through gametophytic pathway in non-treatment were more than those in cold-pretreatment and that the cold-pretreatment increased sporophytic potentiality, whereas inhibited gametophytic potentiality. Thus the effect of cold-pretreatment in anther culture was reported by many researchers.[1,6,8,11]

On the other hand, the change of anther wall tissue was closely related to sporophytic potentiality. The cold pretreatment promoted the browning of anther at early date. The respiratory activity between browned anthers and uncolored ones was not different after 12 days in culture, whereas it was much higher in browned anthers than in uncolored ones after 24 days in culture. This result suggested that browning anther didn't mean a lowering activity and it was related to the structural modifications of anther wall tissue by the uptake of medium components. Sangwan and Comfort[16] explained that the cold stress modifications are more marked in somatic tissue than in the microspores. Liang et al.[11] pointed out that degeneration of anther wall tissue, including tapetum, might be favarable for the anther reaction. However, Tsay[20] reported that the low callus-forming ability caused by anther browning at an early stage in culture was probably due to the production of quinones which are toxic to the microspores.

Thus, the change of cultured anther was closely related to the anther activity in androgenic response. Therefore, we suggested that the pollen viability can be examined with the changes of anther wall tissue and respiratory rate in cultured anther.

摘　　要

벼 葯培養에 있어서 培養葯의 viability의 變化를 觀察하고자 密陽 23號, 大晴벼, 雉岳벼를 材料로 品種 및 低溫前處理, 葯의 褐變에 따른 呼吸率의 變化를 2～3日 間隔으로 調査하였다.

花粉의 發育時期에 따른 培養前 葯의 呼吸活性은 開花直前에 가장높았으며, 培養適期인 stage 3～4에서의 呼吸率은 12.46 O_2 n mole/ml/h/anther이었다.

培養葯의 呼吸活性의 變化는 3~9日頃에 1次頂點을, 9~11日頃에 2次頂點을 나타냈으며, Callus 形成能이 높은 大晴벼에서 가장 높게 나타났다. 低溫前處理는 培養 初期의 呼吸活性을 低下시켰으나 15日頃부터는 無處理區 보다 높은 傾向이었다. 또한 低溫前處理는 葯의 褐變을 促進하였으며, 褐變葯에서 呼吸活性이 높았다.

LITERATURE CITED

1. Chaleff, R.S. and A. Stolarz. 1981. Factors influencing the frequency of callus formation among cultured rice (*Oryza sativa* L.) anthers. Physiol. Plant 51 : 201-206.
2. Chen, C.C. 1982. Toward utilization of anther culture in rice breeding, In Plant Breeding by AAC and ROC. pp 81-86.
3. Chen, Y. 1983. Anther and pollen culture of rice in china. In Cell and Tissue Culture Techniques for Cereal Crop Improvement. Science Press, Beijing, China and IRRI, Manila Philippines. pp 11-26.
4. Chu, C.C. 1978. The N_6 medium and its applications to anther of cereal crops. In Proceedings of Symposium on Plant Tissue Culture. Science Press, Peiking. pp 43-50.
5. Dale, P.J. 1975. Pollen dimorphism and anther culture in barley. Planta (Berl.) 127 : 213-220.
6. Genovesi, A.D. and C.W. Magill, 1979. Improved rate of callus and green plant production from rice anther culture following cold shock. Crop Sci. 19 : 662-664.
7. Honer, M. and H.E. Street. 1978. Pollen dimorphism-origin and significance in pollen plant formation by anther culture. Ann. Bot. 42 : 763-771.
8. Hu, C., S.C. Huang, C.P. Ho, H.C. Liang, C.C. Chang and L.P. Peng. 1978. On the inductive condition of rice pollen plantlets in anther culture. In Proc. Symp. On Plant Tissue Culture, Science Press, Peiking. pp 87-95.
9. Ito, N. 1980. Physiological abnormalities in anthers of sterile-type cool-injury of rice plants. Res. Bull. Hokkaido Natl. Agric. Exp. Stn. 125 : 41-75.
10. Leaver, C.J. 1980. Mitochondrial genes and male sterility in plants. Trends Biochem. Sci. 5 : 248-252.
11. Liang, H.M., H.X. Zhoug and X.Y. Yu. 1980. Preliminary studies on conditions for initiation of dedifferentiation of pollen cells and the change of anther wall tissue in anther cultures. Acta. Phytophysiol. Sin. China 6 : 19-28.
12. Maheshwari, S.C., A. Rashid, and A.K. Tyagi. 1980. Physiology of pollen haploid formation. In Plant Cell Cultures (eds. Sala, F., B. Parisi, R. Cella, and O. Ciferri). North-Holland, Elsevier. pp 393-398.
13. Nitsch, C. and B. Norreel. 1973. Effect dűn choc thermique surle pouvoir embryogéne du pollen de *Datura innoxia* cultive dans l'anthere ou isol'e de lanthere. C.R. Hebd. Séanc. Acad. Sci., Paris 276 : 303-306.
14. Oono, K. 1978. Test tube breeding of rice by tissue culture. Symp. on Methods of Crop Breeding, Tropical Agricultural Research Series 11 : 109-124.
15. Rashid, A. and J. Reinert. 1981. *In vitro* differentiation of embryogenic pollen, control by cold treatment and embryo formation in *ab initio* pollen cultures of *Nicotiana tabacum* var. Vadischer Burley. Protoplasma 109 : 285-294.
16. Sangwan, R.S. and H. Comefort. 1984. Cold-treatment related structural modifications in the embryogenic anther of *Datura*. Cytologia 49 : 473-487.
17. Seo, G.S. 1983. Determination of physiological activity of anthers by ATP test. Kor. Res. Rept. O R D. (S.P.M.U) 25 : 61-64.
18. Sunderland, N. and J.M. Dunwell, 1977. Anther and pollen culture. In Plant Tissue and Cell Culture. (2nd ed. Street, H.E) Blackwell, Oxf. pp 223-265.
19. Toriyama, K. and K. Hinata. 1984. Anther respiratory activity and chilling resistance in