# HELIANTHUS HYBRID AND OBSERVATION OF SOMATIC EMBRYOGENESIS IN THE IMMATURE EMBRYO CULTURE OF SUNFLOWER

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SUMMARY

Sunflower heart-shaped embryos produced from the hybrid embryos of Helianthus annuus L. X H. praecox E. or H. petiolaris N. were cultured in vitro on one modified B-5 medium and formed interspecific Helianthus plantlets on another medium. The plantlets were induced into flowering.

On the other hand, somatic embryogenesis was successful in the immature embryo culture of cultivated sunflower (H. annuus L.). The higher concentrations of sucrose and Zeatin, the higher frequency of somatic embryogenesis induced on the Nitsch basic medium supplemented with 0.5--10.0mg/l Zeatin and 17.5% sucrose. The plantlets were regenerated. Somatic embryogenesis could be also induced on the medium containing 2,4-D 0.5--4.0mg/l but the development and germination of somatic embryos were very poor. The immature embryos produced the embryonic cell groups and embryoids, most of them are emerged in the cotyledon and hypocotyl.Due to degeneration of the surrounding cells of embryonic cell groups and the embryoids, there was the space between the groups and the surrounding tissue.

## INTRODUCTION

The wild Helianthus species are of considerable interest as a source of genetic variation for economically important characters in the improvement of the cultivated sunflower(Helianthus annuus L.). Several attempts to hybridize H. annuus with the other Helianthus species have been made, but most of them with only limited success. Embryo culture has been proposed as a tool for the rescue of the embryo before its abortion. Our overall purpose is to develop such in vitro techniques of embryo culture that any desirable interspecific hybrid within the genus could be produced and applied ( Espinasse, 1985).

In recent years the study on plant somatic embryogenesis has been made great progress. Somatic embryogenesis in application can be conditioned more easily in comparison with organogenesis, the study of which is of important significance on plant tissue culture and cell engineering. Therefore, we have studied some sunflower somatic embryogenesis in the immature embryo culture.

## MATERIALS AND METHODS

"41A", Helianthus annuusL., a male sterility was used as the female parent to cross with the wild male parents which were H. praecox E. and H. petiolaris N. for hybrid embryo culture research. In the research of somatic embryogenesis, we used cultivated sunflower as the two parents.

After pollination for 2--4 days immature seeds were not surface sterilized,directly heart-shaped embryos were excised carefully in the sterile condition, then placed them on the media immediately. The basic Nitsch(1969) and B-5(1968)media

were componded at normal method, and supplemented with various substances in the different experiments. All cultures were maintained at 26°C with a light of 2000 Lux. When the embryos developed and germinated to the plantlets of 3-4 leaves, the plantlets were transplanted in soil with one second sand, exposed to ambient humidity over one week period,then were grown in greenhouse.

For histological studies, the embryos with somatic embryos fixed in Nawaschin solution, dehydrated in an ethanol series, embedded in paraffin wax and cut to 10$\mu$. Sections were affixed to glass slides by heating and stained with hematoxylin for viewing and photoing.

RESULTS AND DISCUSSION

Hybrid Immature Embryo Culture of Sunflower

H. petiolaris N. X "41A" and H. praecox E. X "41A" gave rise to hybrid immature embryos, which were placed on the embryo growth medium containing 1.0mg/l indole-3-acetic acid(IAA). After 2 week culture hybrid immature embryos tended to grow calli mainly. When they were placed on the embryo growth medium containing 0.05mg/l naphthalene acetic acid(NAA), hybrid immature embryos growed embryonically; however, only NAA could not make the immature embryos growth perfectly before germination.For instance, hybrid immature embryos of H. praecox X "41A" were embryonic growth of 71% when the medium contained NAA only; and embryonic growth of 83% when the medium contained 0.05mg/l NAA and 0.01mg/l Kinetin (KT). The results showed that hybrid immature embryos could grow better when they were maintained on the embryo growth medium containing NAA and KT.

Immature embryos could develop and grow perfectly and decrease germination in advance on the medium containing 15% sucrose in two concentrations of 12% and 15% sucrose . The plantlets which were formed from the embryos in 15% sucrose medium were stronger than that of 12% sucrose medium.

The embryo growth medium best suited for hybrid heart-shaped embryos of sunflower as follows: B-5 salts and vitamins,1000mg/l L-alanine, 800mg/l L-glulamine, 160mg/l L-serine, 50mg/l L-tryptophan, 15% sucrose, 0.7% agar, 0.05mg/l NAA and 0.01mg/l KT.

The hybrid embryos germinated, formed the plantlets with 4--5 leaves in the germination medium, then the plantlets were transplanted to sand-soil of the greenhouse. All the hybrid plantlets flowered in the greenhouse during early winter. Some hybrid plants have one or two branches, and shows clearly the character of the wild male parent, because the wild male parent has many branches in the nature. The stems of the hybrid plants are erect stems, that shows the character of the cultivated parent, because the wild parent is stolon, while the cultivated parent is not. Moreover, the leaves of the hybrid are verticillate, but those of cultivated sunflower are alternate.

The immature embryos of which wild species crossed with cultivated sunflower in Helianthus have been cultured to plants in vitro,that shows immature embryo culture on Hilianthus in hopeful

in the sunflower breeding.

Somatic Embryogenesis in Immature Embryo Culture of Sunflower

1. Effect of sucrose concentrations on somatic embryogenesis

When the concentration of Zeatin was 1.0mg/l the white tubercles on the zygotic embryo could be observed in 20% sucrose medium after cultured for 15 days. Observation by the paraffin sections shows that there is a somatic embryo within the white tubercle. After cultured 30 days, in 20% sucrose medium the zygotic embryos of 60% produced somatic embryos; in 15% sucrose medium only the zygotic embryos of 20% produced somatic embryos, that illustrates the high concentration of sucrose promotes the somatic embryogenesis. The sucrose concentration might condition the somatic embryogenesis, too. The results were similar to Litz et al.(1981) report showing.

2. Inducing embryogenesis on Auxin
i) Effect of Zeatin on embryogenesis

Sunflower heart-stage embryos placed on the Nitsch medium containing 17.5% sucrose with different concentration Zeatin. The results are shown in table 1.

Table 1. Effect of Zeatin on somatic embryogenesis in young embryo culture

| concentration of Zeatin (ppm) | No. of embryos | percentage of embryos with embryoids | |
|---|---|---|---|
| | | after 10 days | after 20 days |
| 0 | 40 | 0 | 0 |
| 0.5 | 40 | 0 | 10 |
| 0.7 | 20 | 0 | 10 |
| 1.0 | 40 | 0 | 10 |
| 3.0 | 20 | 0 | 25 |
| 5.0 | 40 | 15 | 25 |
| 7.0 | 20 | 25 | 55 |
| 10.0 | 40 | 25 | 50 |

Somatic embryos within the white, smooth surface tubercles were first observed at the tenth day after culture. The somatic embryos were initially proliferated directly from the middle, surface and terminal portions of the cotyledons and hypocotyls without the formation of a callus intermediate.

Somatic embryos taking higher frequency were formed with higher concentrations of Zeatin.

The somatic embryos from immature zygotic embryos germinated plantlets in the B-5 medium with 6% sucrose.

The experimental results have shown that Zeatin was able to induce the somatic embryos lonely without the other plant hormones. This problem had the contrary reports, as some researchs testified somatic embryogenesis had to be induced by auxin(Zhu C.1978, Evans et al. 1983). Anther proved that the embryogenesis could be induced only by cytomin(Hu S. Y. 1982). Therefore, different species for somatic embryogenesis demand different kinds of inducing substances.

The initial cells for embryogenesis can be told clearly from the cells beside, the initial cell has dense protoplasm, bigger cell nucleus and thick cell wall which isolated to initial cell from somatic cells around. Some initial cells divide continously into the embryonic cell groups which are primordia producing somatic embryos from the observation of the paraffin slides. The cells round the somatic embryo degenerate gradually during the development of the somatic embryo, thus the interspace between the somatic embryo and tissue round it is formed, the culture structure has shown that somatic embryos in the development keep relative independence from the tissue around. The interspace at the structure between the initial cell of a somatic embryo and its round somatic cells is prerequisite for physiological isolation. But there is not all interspace between a somatic embryo and its round tissue, the somatic embryo with suspensor is found in the experiment. Between the suspensor and the round tissue there is no space, that means somatic embryos during the development have to take nutritions and other important substance.

ii) Effect of 2,4-D on embryogenesis

The heart-shaped embryos of sunflower in the Nitsch medium with 17.5% sucrose and 0.5, 1.0, 2.0, 4.0mg/l 2,4-D respectively produced white, smooth tubercles after 20 days. The number of tubercles increased continously untill two month. There in an embryonic cell group which is stained very dark around 2-4 cell layers within the white tubercle by the slide observation. Although the different concentrations of 2,4-D(0.5-4.0mg/l) can induce embryonic cell groups, the groups only proliferate, not develop further.

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