Breeding of Panax ginseng and Plant Tissue Culture

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Abstract

Production of Panax ginseng in Korea has been increased tremendously in the last two decades, the acreage and number of growers having been spread across the country. Improvement of variety, on the other hand, has been seldom carried out during this period, rendering the ginseng growing risky and hazardous.

Breeding work with such perennial crop as ginseng is rather time-consuming, and there are few genesources to start with. Authorities concerned with ginseng production pay very little attention to breeding researches for numbers of reasons. As this crop is cultivated over the entire area of Korea, breeding work cannot be allowed to delay further. In the present article, improvement of ginseng was discussed from the view point of two different categories of breeding concept: (1) conventional breeding and (2) breeding based on the somatic cell genetics.

In the last two decades the number of ginseng growers and its acreage increased tremendously, covering nearly every nook and corner of Korean Peninsula, and the mounting export volume of ginseng and its products is playing an important role in earning the hard currency.

With the rapid increase of ginseng cultivation it is feared that the diseases and insect pests might also spread. When only one variety is cultivated throughout the country, growers would face with many hazards arising from the varied climatic, or biological conditions. When a certain crop is increased in its acreage, first step to be taken is the effort to improve the variety of the crop so that the farmers can grow the plant free of any risks.

In view of the distribution of ginseng acreage, Korea should have developed many varieties by now: varieties resistant to diseases; fit for local environmental conditions; with good marketability, both domestic and foreign; with good root shapes or head sizes; insensitive to sun light; with increased content of specific ingredient. Even in such perennial plant as chrysanthemums, roses, peonies, and orchard trees which need many years for the improvement of variety, thousands of varieties have been

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developed, not to speak of such annual crops as cereals and vegetables.

Breeding work in agriculture is carried out based on the same principles as the biological evolution in nature. The factors concerned with the breeding and evolution are mutation, hybridization and selection. The differences between breeding agricultural crops and evolution of living organisms are that the former is produced by human being while the latter is by the force of nature. New agricultural variety arises in a short period of years while new species in nature is differentiated after a long period of geological age, and in the former case the product is weak needing protection whereas in the latter it is resistant and wild. Breeding is oftern called "controlled evolution". By the process of evolution millions of different kinds of plants, animals and human being were produced from simple microscopic living organisms though it took hundreds of millions of years. These changes of tremendous magnitude in evolution suggest that similar thing can happen in breeding work under man-made environment.

Despite the wide spread of ginseng acreage in this country we have payed so far very little attention on the improvement of variety. Authorities concerned have ignored or delayed the breeding work on the excuse that breeding in this kind of perennial crop is time-consuming and the institutional system is inadequate for the experimental work. Ginseng cultivation in Korea has reached the point where no further delay in the breeding work is allowed.

Ginseng is a perennial crop and it takes two to three years for the plant to reach the flowering stage. This fact along with other disadvantages makes the breeding of ginseng cumbersome and unattractive.

In the present paper the possibility of rapid improvement of ginseng variety is discussed based on two ways of breeding technique: (1) conventional breeding and (2) somatic cell genetics.

Ginseng Breeding by Means of Conventional Method

Hybridization is a representative of breeding technique and majority of present-day varieties in crops, ornamental plants and orchard trees are the products of this mothod. But as far as ginseng breeding is concerned, hybridization is most unlikely to be taken up because ginseng variety has not been differentiated at present not only in Korea but also in other countries. This means that in ginseng there exist very few known gene sources to be used in hybridization.

Ginseng is self-fertilizing but considerably out-crossing seed-propagated plant. In

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the ginseng farm it is expected that the population contains countless numbers of variants with desirable morphological, ecological or physiological characters for yield, quality, resistance to diseases, and root components. Some of the characters may be of micro or invisible, while others may be of macro or visible. Ginseng grower, if he is interested in the breeding work and makes careful observation of his field, would select out numbers of lines with valuable agronomic characters from the population. Even the invisible characters can be selected with relative ease due to the improvement of screening techniques. This kind of line separation method can be achieved comparatively in shorter period as compared to the hybridization breeding.

Ginseng Breeding by Means of Somatic Cell Genetics

In 1940's pioneering studies of Beadle and Tatum on the mold Neurospora including induction of biochemical mutants led to the development of microbial genetics. The wild type Neurospora is able to synthesize all of its cellular constituents except biotin from carbohydrate and inorganic salts. Mutants are incapable of performing certain biosynthetic steps and can grow only if a specific growth factor is supplied in the nutrient medium. The technique and knowledge obtained from the selection of biochemical mutants in microorganisms could be applied in the breeding of higher plants.

In higher plants it has been possible to induce callus mass from the differentiated somatic tissue, cutlure the callus cells as single cell population in liquid medium and finally redifferentiate adult plants from the cultured single cells. Moreover, haploid cell population could be obtained from the haploid callus or plants originated from the microspores of higher plants. These haploid cells when transferred to the differentiation medium can regenerate haploid plantlets. As the haploid cells has n nuclear phase, mutant characters can be expressed directly when treated with mutagens as in the case of the induction of biochemical mutants from the molds. In this way in higher plants mutant cells resistant to diseases or cells with higher content of certain component can be produced, ^{2,9,10,16)} thus enabling the plant breeding to carry out at cell levels.

Plant cell clones have been recovered which are deficient in the synthesis of amino acids and other basic metabolites, or are resistant to drugs, such as streptomycin, or to toxic compounds including amino acid analogues and bacterial toxins¹⁶. Among this latter class of mutants, Carlson² recovered mutants from mutagenized tobacco cell cultures which were resistant to the glutamine analogue, methionine sulfoximine.

This compound has structural similarities to the bacterial toxin produced by the pathogen, *Pseudomonas tabaci*, which causes wildfire disease of tobacco. Plant regenerated from these mutant cell lines has an increased level of resistance to the bacterial pathogen.

One of the disadvantages in plant breeding is the long period of years needed until the release of new variety. Nowadays the science and technology are progressing with rapid pace. Accordingly our taste for food, cultivation technique and struture of agriculture are also undergoing rapid changes. Ten or so years of breeding period is too long under such quickly changing situation. New variety developed may turn out to be useless and has to be discarded. The other defect of plant breeding is the fact that it needs acres of farm land and in open field the right season for cropgrowing is restricted.

These disadvantages of conventional breeding can be overcome by using millions of *in vitro* cultured suspension cells and by repeating the experiment all the year round in laboratory. This kind of breeding by somatic cell genetics is particularly fit for such crop as ginseng which is perennial and whose major breeding purposes are increment of certain component or resistance to diseases.

Viable protoplasts of higher plants have now been isolated from a large number of species with the aid of cell-wall degrading enzymes isolated from several different microorganisms. These naked protoplasts can reform cell-wall, fuse each other, and regenerate mature plants.^{4~8},^{11~13},¹⁵⁾

With the elimination of the cell-wall obstacle, these protoplasts should be ideal receptors for foreign genetic material. Naked protoplasts take up such foreign micro-structures as DNA, virus, or bacteria. Although it is not certain that whether the foreign DNA is integrated into the genome of the recipient species or it can express its genetic information, ¹⁴⁾ the facts that protoplast can be isolated and it can regenerate adult plant are remarkable progress in the genetic manipulation at cell level of higher plant. Someday it may be possible that protoplasts of ginseng take up DNA concerned with synthesis of certain valuable component and they redifferentiate full plants, thus bringing about a new type of ginseng plant.

Most genes are grouped together in large chains known as chromosomes. A few, however, are combined in small rings known as plasmids. Techniques have been developed for removing plasmids from their host bacteria, spliting them open chemically, and inserting new genes into them. The new plasmids thus created can then be introduced into other bacteria, where the genes start to change the hereditary

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characteristics of their new host bacteria just as if they had been there all along.³⁾ Hybridization at the DNA level is possible not only among bacteria but also between bacteria and animals or between bacteria and higher plants. If these techniques can be adoted to plants, then specific desirable genetic information, such as the genes for nitrogen fixation, might possibly be transferred from nitrogen fixing bacteria to plant ^{1,14)}. If such non-leguminous crops as corn or rice could be given the genetic ability to fix the aerial nitrogen, this could dramatically reduce the dependence on expensive fertilizers for food production. One of the advantages of the breeding by means of genetic manipulation at cell level is that it can be accomplished in far shorter period as compared to the conventional breeding method.

Breeding of Korean ginseng is an urgent problem which must not be delayed further under the pretext that it needs so much years or that experimental institution is inadequate to do that work. Every means available has to be worked out to achieve the rapid improvement of ginseng variety.

Literature Cited

- Cannon, F. C., Dixon, R. A., Postgate, J. R., and Primrose. S. B.: Chromosomal integration of Klebsiella nitrogen fixation genes in Escherichia coli. J. Gen. Microbiol. 80: 227-239 (1974).
- Carlson, P. S.: Methionine sulfoximine-resistant mutants of tobacco. Science 180: 1366-1368 (1973).
- Chang, A. C. Y., and Cohen, S. N.: Genome construction between bacterial species in vitro: Replication and expression of Staphylococcus plastid genes in Escherichia coli. Proc. Nat. Acad. Sci. (USA) 71: 1030-1034 (1974).
- Cocking, E. C.: A method for the isolation of plant protoplasts and vacuoles. Nature 187: 962-963 (1960).
- 5. Grambow, H. J., Kao, K. N., Miller, R. A., and Gamborg, O. L.: Cell division and plant development from protoplasts of carrot cell suspension cultures. *Planta* (Berl.) 103: 348-355 (1972).
- 6. Harn, C. and Choi. C. K.: Studies on the fusion of rice protoplasts. Kor. J. Bot. 15 (4): 13-17 (1972).
- 7. Harn, C.:. Plant protoplast culture and somatic cell hybridization. Kor. J. Bot. 15(3): 84-88. (1972)
- 8. Harn, C. and Kim, M. Z.: Fusion of protoplasts isolated from mesophyll cells of N. rustica. Korean J. Breeding 5: 1-4 (1973).
- 9. Maliga, P., Sz-Breznovits, A., and Marton, L.: Streptomycin-resistant plants from callus culture of haploid tobacco. *Nature New Biol.* 244: 29-30 (1973).
- 10. Maliga, P., Marton, L., and Sz-Breznovits, A.: 5-Bromodeoxyuridine-resistant cell lines from haploid tobacco. *Plant Sci. Letters* 1: 119-120 (1973).
- 11. Otsuki, Y. and Takebe, I.: Isolation of intact mesophyll cells and their protoplast from higher plants. *Plant and Cell Physiol.* 10: 917-921 (1969).
- 12. Power, J. B., and Cocking, E. C.: A simple method for the isolation of very large numbers of leaf protoplasts by using mixtures of cellulase and pectinase, *Biochem. J.* 15:

Proc. Biochem. Soc. 33 (1969).

- 13. Ruesink, A. W. and Thimann, K. V.: Protoplasts: Preparation from higher plants.. Science 195: 280-281 (1966).
- 14. Scowcroft, W. R.: The potential use of somatic cell genetics in plant improvement. Sabrao J. 7: 147-158 (1975).
- 15. Takebe, I.: Protoplasts from higher plants: A new experimental system for cell biology. Symposia Cell. Biol. 22: 205-212 1971.

고려 인삼의 육종과 조직배양

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초 록

최근 20년동안에 인삼의 경작자와 재배면적이 급증, 거의 전역에 재배되고 있고 이. 작물이 한국경제에 미치는 영향도 크다.

한편 재배지역의 증가에도 불구하고 인삼의 육종은 거의 도외시되어 왔고 품종의 분화가 전혀 안되어 있다. 기후, 토양 및 생물학적 조건등이 지역마다 다른데 단일 품종을 장려할때에 경작자들은 병충해를 위시해서 많은 재난의 위협하에 놓이게 된다. 한국과같이 인삼이 중요한 위치를 점유하고 있는 나라에서는 벌써 형태 생태 생리적 품종의분화가 있었어야 했고, 내병성, 특이성분 등에 관한 품종들이 다수 만들어 졌어야 했다. 「인삼은 다년생이고 육종에는 불편한 점이 많아 개량이 힘든다. 또 육종을 하기에는 시험기관의 제도적 애로도 많다」등의 구실하에 육종을 소홀히 하기에는 재배지역이. 너무나 광범화되었다.

본문은 인삼의 품종을 단기간에 개량하는데 있어 (1) 현행의 고전 육종법을 이용하는 길과, (2) 조직배양에 의한 신 육종법을 쓰는 두 길이 있음을 제시한 것이다.