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Combination of Biotechnology and breeding methods for creation of potato varieties resistant to abiotic and biotic factors

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Abstract

The article reviews the research on the use of cell selection methods for creating original breeding potato material resistant to biotic and abiotic factors. Culture media using selective factors, cultivation conditions have been modified and a scheme of cell selection has been developed. It is established that using cell selection, the stepped introduction of selective factor in the culture medium of culture in vitro is effective. The working concentration of a selective factor - polyethylene glycol (PEG) at the stage of callus tissue is 5%. After culture stabilization during the passage without a selective factor callus tissue is advisable to be exposed to high temperatures. Processing at high temperatures was started at the stage of morphogenesis in the range (30-50^0 C) with an increase up to 5^0 C in each passage. Calluses stood high temperatures for 7 days. To study the mechanisms of host-pathogen interactions and the development of screening systems of resistant variants against phytopathogens the use of salicylic acid as a selective factor in cell cultures and tissues is demonstrated. We have proved to observe morphoregulatory and cytotoxic effects in a heterogeneous suspension culture in vitro when added to the nutrient medium of salicylic acid. We have obtained cell lines that are resistant to selective factors. The article reviews the questions of potato breeding for resistance to adverse conditions, including drought and heat resistance. The offspring at the early stages of breeding and their best genotypes, brought to the competitive test, have been assessed. On the basis of research and scientific development conducted a number of scientists have obtained the information on breeding stages for drought resistance and heat resistance as the elements of adaptability.

Keywords: abiotic, biotic factors, selective factor, resistance

Introduction

Numerous biotechnologies for potato genetics and breeding have been created owing to high plasticity in the culture in vitro. The use of such technologies allows obtaining material with a unique combination of genes.

Culture in vitro provides for experimental impact directly on the somatic cell and totipotency property allows obtaining variable forms from altered cells. The cell population, which is characterized by physiological, cytological and genetic heterogeneity, is formed with further cultivation based on artificial nutrient media as a result of induction of proliferation.

When applying cell culture and tissue we may receive an average of about 3% of potato self-clones, useful for breeders (Karp et. al., 1989).

The search for the positive deviations can be made more oriented, if the population of isolated protoplasts, cells or callus explants is to be cultivated in the selective conditions. Thus most of the cells die, and only those cells that are resistant to this factor survive. After testing for stability of the characteristic (long-term cultivation) in selective and non-selective conditions, we may assume that the characteristic is genetically conditioned. Subsequent regeneration allows obtaining plants resistant to this stress factor (Zakharchuk, et al., 2001; Stolle, et al., 1984).

Cell selection for heat and drought resistance

In order to imitate in vitro stress effect of drought, the nutrient media, supplemented with osmotically active substances that reduce external water potential, can be applied. Polyethylene glycol (PEG), which is an osmotically active substance, does not penetrate into the cell, was used as a selective agent for breeding of resistance to drought. The first information on the allocation of tobacco cell lines resistant to stress induced by PEG appeared in 1979 (Dragiiska, et. al. 1996). Later R. Bressan with the coauthors (Dragiiska, et. al. 1996) used a line of tomatoes that were subjected to water stress by culturing in the presence of PEG for breeding for drought resistance. Selective nutrient medium for callus cultivation contained 15% of PEG 6000. As a result of research callus resistant lines were obtained.

The aim of our work is to find new effective methods for drought-resistant and thermoresistant forms of potatoes using the cell selection method.

In the research 6 varieties of potatoes of the breeding of the Institute for potato research of the National Academy of Agrarian Sciences of Ukraine were used: Zov, Dovira, Levada, Melodiya, Palitra, Slovianka. Fragile callus tissue was obtained from leaf and stem explants of plants in vitro. Suspension culture was obtained by transferring undifferentiated fragile callus into liquid nutrient medium MC (Adamovskaya & Molodchenkova, 1997) and grown on rocking. To obtain potato cell lines the suspension culture was mixed with agar nutrient medium at a ratio of 1/5 and sown in Petri dishes. At this stage selective factors were injected in the culture medium. With each passage of the culture the concentration of selective factors was increased. The colonies were transplanted to the medium for growth, morphogenesis and regeneration. After callus reached sizes of 8-10 mm they were used in further studies of cell selection. The number of planted calluses for each variety amounted to 100 explants.

Organized development in vitro occurs due to a specific interaction of an explant, culture medium and cultivation conditions.

Stem and leaf explants were cultivated on medium Murasihe and Skoog in the presence of auxin 2.4-D (2.0-5.0 mg / l). Explants were cultivated in the dark at 25^0C for 15 days. The stems of explants on the 8^{th} day of cultivation showed intensive growth of callus tissue. Well-developed callus fragile tissue was transplanted to the medium for suspension culture. The cell culture was grown in the dark on rocking at 25^0C, humidity 80-85%. In 3-4 weeks suspension culture has density of $10^{-4} – 10^{-5}$ of cells per 1 ml and light colour. Even finely dispersed suspension of potato cells has 50-60% of heterogeneous cell aggregates from tens to hundreds of cells. Therefore,

to stabilize the suspension culture 2-3 passages in liquid nutrient media, reducing the concentration from 2.4-D to 0.5 mg / l, were carried out. Further the suspension culture was transferred to agar nutrient medium for callus growth. Auxin was excluded and 6-benzylaminopurine was injected in the concentrations - 0.5; 1; 1.5; 2 mg/l (for the cells from the stem explants) and 0.1; 0.5; 1 mg/l (for the cells from the plate explants).

Potato breeding was started at the cellular level to test the selective concentration within which different cell viability is manifested. PEG with a molecular weight of 8000 was introduced into the nutrient medium in concentrations of 0.5-5%. It should be noted that low concentrations of PEG caused stimulation of weight gain of callus tissue, increased - necrosis and growth inhibition. Selective factor was introduced into the medium, step-by-step increasing concentration in each one, or through one passage up to 0.5%. Thus the stabilization of the growth of callus culture occurred with subsequent selective breeding.

The effect of different concentrations (PEG) and high temperatures was shown in browning of callus tissue, reducing the number of viable cells, reducing or lack of weight gain.

In the control (without selective factor) calluses of the varieties were from pale to green colour and had smooth structured surface, callus tissue growth amounted to 5 mm.

Using different concentrations of PEG, we concluded on a stepwise introduction of selective factors. Working concentration at the stage of callus tissue is 5%. Upon stabilization of the culture during the passage without selective factor we additionally began to expose callus tissue to high temperatures. We began the exposure to high temperatures at the stage of morphogenesis in the range (30-50^0 C) with an increase up to 5^0 C in each passage. We observed a negative impact of high temperatures on cell growth (reduction in the number of viable cells and increase in brown callus tissue mass).

At this stage of the research we obtained cell lines that are resistant to selective factors.

Salicylic acid - inductor of resistance to Fusarium dry rot agent

In recent years, the problem of potato yield losses during storage caused by Fusarium dry rot lesions has significantly worsened that has set a new task before the breeders, biotechnologists, geneticists to develop new varieties and obtain the original forms resistant to this pathogen. The success of breeding activity in this area depends on the effectiveness of methods for determining the reaction of different genotypes to pathogen penetration and formation of mechanisms of resistance at different stages of systemic interaction plant-pathogen.

The studies have shown that a significant role in these processes belongs to the system protease-inhibitors (Keller, nd). Peptidohydrolases of plants are involved in the regulation of intracellular metabolism, particularly, in the formation of mechanisms of adaptation to adverse factors of different nature, including resistance to phyto-diseases. It is now believed that increasing the level of inhibitors of proteolytic enzymes is one of the defense mechanisms of plants in response to infection.

Among the chemicals that activate plant defense mechanisms, the most well

-known is salicylic acid (Oleynik, 1997). It is shown that salicylic acid serves as a system signal and an inductor of hypersensitivity reaction and formation of resistance to a number of phyto-diseases, including Fusarium dry rot.

The study of the influence of salicylic acid on callus and suspension culture of potato and identification of optimal concentrations of a selective factor for subsequent obtaining of the forms resistant to phyto-diseases, particularly, to Fusarium dry rot, was conducted on potato varieties of breeding of the Institute: resistant to Fusarium - Slovianka, Serpanok; susceptible - Nezabudka.

Callus culture was obtained using the leaves and stems of test tube plants of initial varieties, which were cultured in Petri dishes in the media to produce fragile and morphogenic callus.

The obligatory condition of dedifferentiation of plant cells is the presence, except for the fertilizer element, of hormonal factors in the medium. Induction of callus creation and callus growth was studied on the media containing different growth regulators. We have tested the effect of 2.4-D and kinetin on the intensity of callus formation in the range from 1 to 5 mg/l. As a rule, callus of all varieties are well developed at a concentration of 2.4-D in the range of 2-5 mg/l, the average percentage of callus formation on leaf explants was equal to 75%, on stem explants - 90%. It should be noted that calluses grew in the form of dense mass at low concentrations of 2.4-D (1-3mg/l), and they were fragile at a concentration of 4-5 mg/l. However, according to our previous studies, regeneration is possible only on solid calluses when removing calluses in the medium for morphogenesis.

Thus, these data indicate genotypic conditionality of the processes of growth and development in culture in vitro, thus, optimizing the cultivation conditions, we can identify the potential features of genotype to callus- and morphogenesis. These works are particularly important to be carried out previously before the beginning of a passage of callus clones in a selective medium to determine the initial stress load of a nutrient medium for a particular genotype.

Suspension culture is initiated from fragile calluses in the medium M-C and cultured with constant shaking in flasks at 25 ± 1 ° C, relative air humidity 80-85%, in the dark.

Previously obtained calluses were transferred to the medium for morphogenesis, and then globular calluses with fragile structure were selected for obtaining the suspension. To transfer in a liquid medium morphogenic parts of 1-2 g were extracted from such calluses and brought into the flask with 30 ml of medium.

Medium of M-C with 2.0 mg/l 2.4-D, 0.2 mg/l of kinetin and 0.1 mg/l of gibberellic acid was used. Subcultivation was conducted twice a week. In 3-4 months shallow aggregated morphologically homogeneous suspension, active in growth, formed from a cell of meristem type with dense cytoplasm type and thin cell wall, was received.

Introduction of selective agents to the nutrient medium provides for an opportunity to study their effects on the cellular level and if the reaction is adequate, it is possible to use a suspension culture as a test system for the evaluation of genotypes on resistance to adverse conditions (Oliynik, 1998a, 1998b, 1998c, Oliynik, 2001).

Growth of heterogeneous suspension can be characterized by various parameters relating to an aggregated fraction. Visual assessment (necrosis, cell viabil-

ity) was conducted for this purpose.

Addition of salicylic acid (0.5, 1.0, 2.0, 3.0, 5.0 mM/l) caused inhibition of suspension culture growth, necrosis was noted. Necrosis was weaker with 0.5-1.0 mM of salicylic acid. We should note that the differences were more vividly manifasted in a state of suspension and callus tissues at high concentrations (3-5 mM/l). When the exposure was increased, the differences strengthened. Visual assessment of suspension culture necrosis indicates its growth with increasing concentration of salicylic acid and exposure and specificity of varieties at low and medium concentrations of the studied genotypes. The use of increasing concentrations allowed retaining higher levels of growth of suspension cultures at high concentrations of a selective factor and at the same time weakening the influence of genotypes' adaptive capacities.

It should be noted that a significant measure using salicylic acid to assess the genotypes to Fusarium dry rot is to increase the level of 2.4-D to a level of 5.0 mg/l, since at lower concentrations we observe rapid rhizogenesis that masks the toxic effect. This suggests that one of the mechanisms of host-pathogen in the system of F. solani is a hormonal imbalance.

Changing the selective medium (2.0 mM/L, exposure 4 weeks) to a control one, we observed recovery of viable cells (for the varieties Nezabudka, Serpanok, Slovianka, respectively, 276%, 205%, 122% of the original, which is fixed to the transfer of cultures on selective medium).

As a result of studies we found the best-critical concentration of salicylic acid as a selective factor that was 3 mM/l of the medium and stable cell lines were obtained.

Further studies will be directed to obtain stable potato plants and study the expression of resistance feature at the level of the whole plant.

Potato breeding for resistance to adverse conditions

One of the main measures to improve the yield of potatoes is the creation and introduction of varieties adapted to the growing conditions. However, different soil and climatic conditions and climate change (spring and summer drought, high temperature) lead to a significant reduction in productivity, promote greater affection of plants to diseases (Osypchuk, 2009)

In some years the soil temperature during the hot summer months is so high that the formation and growth of tubers is suspended and resumed during cool weather, if moisture is sufficient.

Excessively high temperature and soil drought can cause wilting and suspension of growth of the whole plant. This is manifested in the formation of shallow and deformed tubers with growth, cracking, hollow formation, darkening of the pulp.

Therefore, an important task of potato breeding is to create varieties adapted to adverse environmental conditions, the main of which are drought and heat resistance.

It should be noted that the drought resistance of plants is the ability to adapt to drought and implement growth, development and reproduction during ontogenesis, owing to the properties that arise in the course of evolution under the influence of the conditions for the existence (Genkel, 1956)

Adaptability as both heat and drought resistance are related to the ability to provide high and stable yield in different environmental conditions. It can be adjusted in adverse conditions through the induction of physiological and biochemical processes at the level of formation of the phenotype within normal range of the reaction to these conditions (Zhuchenko, 1999).

It is found that the most important indicators that characterize drought resistance of plants is root system maturity and depth of its penetration into the soil, degree of leaves' wilting and rate of turgor recovery, temperature of protein coagulation in young leaves during coming-up and flowering.

The ability to separate potato leaves to stand a relatively long period of dehydration and quickly restore the turgor may indicate relative drought and heat resistance (Filippov, 1964).

In Kazakhstan, drought and heat resistant varieties Alatau and Vevi are allocated on challenge backgrounds, temperature of protein coagulation of which was 70 -75^{0}C (unstable sort Zorka – 50^{0}C) (Moshnyakov, 1997). The criterion for drought resistance is a higher plant productivity in the conditions of insufficient water supply and high temperature of air and soil (Genkel, 1956).

Several forms of S.andigenum type, as well as S.molinae type (Bukasov, 1959) are characterized by heat resistance, which can be explained by a more developed root system and lower leaf surface of strongly dissected leaves.

Factors that increase drought and heat resistance also include anatomical and morphological features: bush habitus, type of presence of leaves (stem or leaf). Upright bush, which has a weak presence of leaves, promotes rapid soil drying, which is beneficial for plant resistance to diseases, but not always to drought and heat. And the more beneficial is an intermediate type of presence of leaves with highly dissected leaves.

Conclusion

The aim of our research was to assess and identify the combinations of crosses in the first tuber generation without manifestation of external defects on tubers: cracking, cuts and growth and bringing the selected genotypes to a competitive test, evaluate them in the dry year, outline the stages of selection to create drought-resistant and heat-resistant varieties.

Populations of first tuber generation were obtained and studied by selection schemes (Nemishaieve, 2002). At all stages best selection numbers were studied and selected for the complex of features. Those combinations, among which the best genotypes were brought to the competitive test, are marked, including high yield in the dry year. Based on the experiments and analysis of scientific developments conducted by a number of prominent scientists the selection stages to create heat and drought-resistant varieties have been outlined.

Among the combinations studied in the first tuber generation we have detected 18-29% genotypes with the yield of 750-1000 g/bush without cracking and cuts on the tubers - 65-78%, and without tuber growth - 69-82%, with a combination of stated features - 6-14%. According to the results of years long research we selected the genotypes that were studied in the competitive test in combination Bahriana x Bellaroza - 4 genotypes; Slovianka x Bellaroza - 3; Slovianka x Dina - 2; Barylchyk-

ha x Tyras - 2; in other combinations - 1 genotype. These genotypes withstood the action of negative factors including high temperatures and drought at the stages of the selection process, so they can be considered to be relatively resistant to these factors. Among them early varieties - H.06.88-5 (Svitanok kyivskyi x Biluha) and H.06.30-4 (Slovianka x Dina); middle-early - H.07.105-2 (Bahriana x Zdobutok); mid-season - H.05.6-10 (Barylchykha x Tyras), H.07.56-4 (Slovianka x Dina); H.07.55-21 (Slovianka x Bellaroza).

In dry 2012 at the end of the growing season according to the yield we highlighted the selection number H.06.30-4 (Slovianka x Dina) - 279 kg/ha of tubers that is 27 kg/ha more than Tyras and 104 kg/ha more than Serpanok. This variety didn't have cracks, growth and cuts on tubers. The bush is half-spreading, intermediate type of presence of leaves.

Early selection number H.06.88-5 (Svitanok kyivskyi x Biluha) according to the gave way to the standard variety Tyras, but 62 kg / ha more than standard variety Serpanok. We found 0.2% of tubers with cracking, although growth and cuts were not detected. It has a half-spreading bush and leaf type according to the presence of leaves.

In the middle early group according to the yield we highlighted the selection number H.07.105-2 number (Bahriana x Zdobutok) - 235 kg/ha (77 kg/ha more than standard variety Svitanok kyivskyi). Here we found 2% of tubers with cracking, growth and cuts on the tubers were not found. It has an upright bush and an intermediate type of presence of leaves.

In the mid-season group according to the yield we highlighted the selection number H.05.6-10 (Barylchykha x Tyras) – 275 kg/ha (29 kg/ha more than standard variety Slovianka and 45 kg/ha more than standard variety Yavir). We have found 0.5% of tubers with cracking, tubers with growth and cuts were not found. It has an upright bush and an intermediate type of presence of leaves.

A mid-season selection number H.07.55-21 (Slovianka x Bellaroza) provided for the yield of 318 kg/ha (72 kg/ha more than standard variety Slovianka and 88 kg/ha more than standard variety Yavir). It has an upright bush and a stem type of presence of leaves.

Under the results of our research the stages to create drought-resistant and heat-resistant varieties have been outlined:

1. Selection of breeding pairs for hybridization is to be carried out with regard to their interspecific origin with well developed root system.

2. Conduct of breeding material analysis during the second digging down in competitive test on the development root system.

3. Evaluation of selected genotypes in the south of Ukraine.

4. At the stage of selection special attention to be paid to the evaluation of varieties and breeding material in dry and high temperature years.

5. Factors that increase drought and heat resistance should include anatomical and morphological features: bush habitus and type of presence of leaves (stem or leaf). In this case the more important is an intermediate type of presence of leaves with highly dissected leaves.

6. Application of biological assessment methods in breeding for specified indications.

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