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THE MANIFESTATION OF PROPERTIES IN DISTANT HYBRIDS OF *RIBES* L. AND *GROSSULARIA* MILL. (= *RIBES UVA-CRISPA* L. SUBSP. *RECLINATA* (L.) RCHB.) WITH DIFFERENT GENOMIC COMPOSITION

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ABSTRACT

The results of long-term experiments to study the development of traits and biological features at the distant hybrids currants and gooseberries with a different genomic composition. It was established that amfihaploidy differ from the original parental forms with character of growth and color shoots, close fitting bud palet, bud shape, the size of leaves, inflorescence, flowers in the flower racemes. It was determined the possibility of using allotriploidy forms as an intermediate in the preparation of allotetraploid and fertile diploid recombinants with economically valuable traits. It was identified that some forms of amphidiploids have the complex shapes immunity, increased winter hardiness, fruits with large and small seeds.

Key words: currants, gooseberries, breeding, polyploidy, amfihaploid, allotriploid, amphidiploids

INTRODUCTION

Among fruit and berry plants which are cultivated in Belarus, currant (*Ribes nigrum* L.) and gooseberry (*Grossularia reclinata* Mill. [= *Ribes uva-crispa* L. subsp. *reclinata* (L.) Rchb.]) berry bushes are important. Their berries are rich in a valuable set of vitamins, minerals, and enzymes and play an important role in a balanced diet, prevention, and successful treatment of many human diseases.

Gooseberry and currant have not only a number of valuable economic characteristics but also some shortcomings preventing them from wider introduction in agricultural production. The possibility to create forms based on the hybridization and combining the best features of currants and gooseberries, which are deprived of their shortcomings, offers great opportunities in the selection of these crops for increasing the production of multi-vitamin products.

In spite of the progress made on creation and study of distant reciprocal *Ribes* x *Grossularia* hybrids (Bavtuto 1980, Dubrovski 2012, Knyaseva 2004, Sankin 1993, Chuvashina 1980), there are no data on the manifestation of the whole complex of properties of distant hybrids with different genomic composition.

In this respect, the aim of our research was to study the manifestation of the properties and biological features in the distant hybrids of currant and gooseberry with different genomic composition.

MATERIALS AND METHODS

Hybridization of black currant (*R. nigrum* L.) with gooseberries (*Gr. reclinata* Mill.) at diploid and tetraploid levels, reciprocal crossings of tetraploid forms with the original diploids, and backcrossings of the obtained tetraploid hybrids of *R. nigrum* x *Gr*. *reclinata* tetraploid with the original tetraploid forms allowed us to obtain plants with various combinations of the genomes of original forms. The objects of study were 16-chromosome amfihaploids (Ag) with the genomic composition of BG and GB, 24-chromosome allotriploids (Td) with the genomic composition of BGG and BBG, 32-chromosome allotetraploids (At) with the genomic composition of BGGG and GBBB, and 32-chromosome amphidiploids (Ad) with the genomic composition of BBGG in our selection (the symbol of the black currant genome is «B» and gooseberry genome is «G») (Buchenkow 2012, Buchenkow 2013).

Cytological analysis and chromosome calculation was carried out on permanent and temporary samples obtained by the standard technique of cytological research (Ribin 1967).

Self-fertility was determined in per cent, and it was based on the results of the fruit inceptions at self-pollination of flowers under the insulator.

The immunity study was performed in the conditions of natural plant infection with pathogens (%) or insect damage (points).

Winter tolerance was measured with a 5-point scale field method; the principle of the method was in the annual recordings of the degree of shoot freezing.

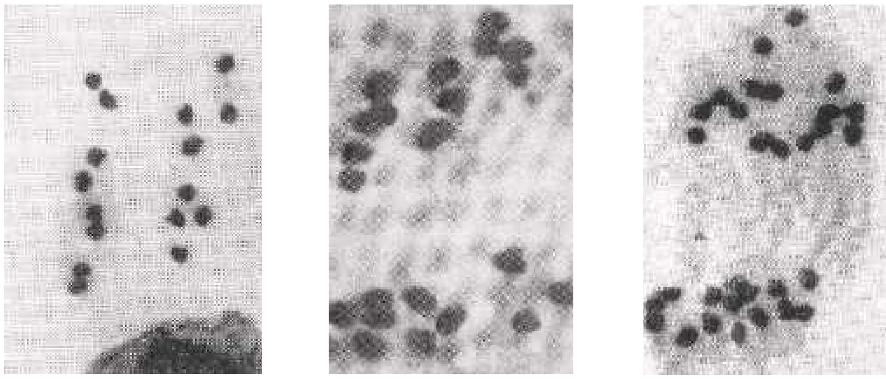
The quality of the pollen was determined by its germination in wet chambers on the medium consisting of agar-agar and 10% sucrose

Field experiments, observation and description of the properties were carried out according to the Program of the study of fruit, berry and nut crops (Ogolcova 1999).

RESULTS AND DISCUSSION

The combination of *Ribes* and *Grossularia* genome in these hybrids can be represented as 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1, 2 : 2. A comparative study of distant hybrids with different genomic structure has allowed us to establish the patterns of inheritance and feature display in F1 in the combination depending on the number of genomes of parental forms (Fig. 1).

Ag, combining the equal number of currant and gooseberry (BG) chromosomes, are distinguished in intermediate nature of the inheritance of the original parents and



a b c

Fig. 1. Chromosomes of *R. nigrum* x *Gr. reclinata* (anaphase I of microsporogenesis): a – amfihaploid (BG), b – allotripploid (BVG), c – amphidiploid (BBGG)

Source: own research

a number of new structures; among them the suppression of such a feature as the amount of burrs of the shoots is important. Such features of currant as crinkles of the upper side of the leaf, pubescence of a flower raceme, and brownish shade of the shoots which are completely suppressed by doubling the number of gooseberry chromosomes and are manifested as dominant features in Ag .

The increase in the number of gooseberry chromosomes from 8 to 16 also affects the nature of the manifestation of other features in F_1. Thus, Td (BGG) is characterized by the intermediate type of inheritance of such a trait as the amount of burrs: small burrs are formed only on young shoots, placed sparsely and disappear quickly.

Amplification of gooseberry characteristics with the increasing number of its genomes affects the structure of a flower raceme. So, Ag raceme is 6,1 ± 0,2 cm (currants have 7,1 ± 0,3 cm and gooseberries have 1,4 ± 0,7 cm) and carries 4 ± 2 flowers in average (the number of flowers in currant racemes does not exceed 8 ± 3 as for gooseberry, this number is 2 ± 1). Outstretched raceme position in Ag refers to new structures compared with a down-curved raceme of currants and gooseberries. Td is characterized by short (1,2 ± 0,7 cm) down-curved raceme with 2 ± 1 flowers. The flowers of Td are larger than Ag flowers (the flower length of Td is 8,5 ± 0,5 mm, when Ag flower has the length of 7,5 ± 1,5 at; Td flower diameter is 9,5 ± 0,5 mm, when Ag's is 8,5 ± 0,5), which gives them a resemblance to the gooseberry flower.

The differences among Ag and Td in the inheritance of structural features of the parts of the flower are marked. Ag sepals are often narrowly lanceolate (the currant property) with a wide range of variations. Td sepals and petals are similar to the gooseberry. Some properties of the gooseberry are inherited as a dominant in Td and Ag, i.e., the truncated tip of the sepals and petals and the bent position of sepals.

The feature of sepal coloring appears especially in F1 hybrids. The feature of green colored sepals is completely suppressed if there is one gooseberry genome. *Ribes*-colored sepals are dominated as the green color is likely to be determined by the recessive genes. By doubling the number of gooseberry chromosomes, this feature is not fully suppressed, and the sepals are of mixed red-yellow-green color.

The doubling of gooseberry genome also causes the dominance of a number of features typical for gooseberry, i.e. the shape of the bush, the color and the character of the surface of the shoots, the position of the gemmae on the shoots, the form of the leaf base and dense fluffing of its lower surface, the absence of white tips on the serratures on the leaf edge and ethereal glands, pinkish flowers, ribbed ovary, duality and the stile pubescence.

Thus, doubling the number of gooseberry genomes in hybrids F1 *R. nigrum* x *Gr. reclinata* enhances the manifestation of its properties. This turns up in changing in the size of vegetative and generative organs of the hybrid: the gemmae, flowers, sepals and petals, stiles, ovary, flower racemes, and the number of flowers in a raceme, i.e., quantitative traits that have probably polygenic type of inheritance. At the same time, doubling the number of gooseberry genomes of *F1 R. nigrum* x *Gr. reclinata* hybrids leads to inheritance a number of alternative qualitative features: the shape and color of gemmae, leaf laminae, the petals and sepals, and the time required for phenophases. To dominant features manifested regardless of the number of genomes of original forms, sprawling types of bush, high winter tolerance, and the immunity should be included.

A number of new structures arising from Ag, does not disappear in Td with doubling the number of gooseberry chromosomes. These structures include the heterotic type of the bush, leaves varying in shape and color, the shape and the length of hypanthium, and in the length and the width of the sepals. Consequently, somatic (powerful bushes, large shoots, leaves, and gemmae) and reproductive (large flowers) heterosis are manifested in Td, as well as in Ag. Unpretentiousness of the hybrids to the growing conditions and handling, immunity and winter tolerance can explain the adaptive heterosis.

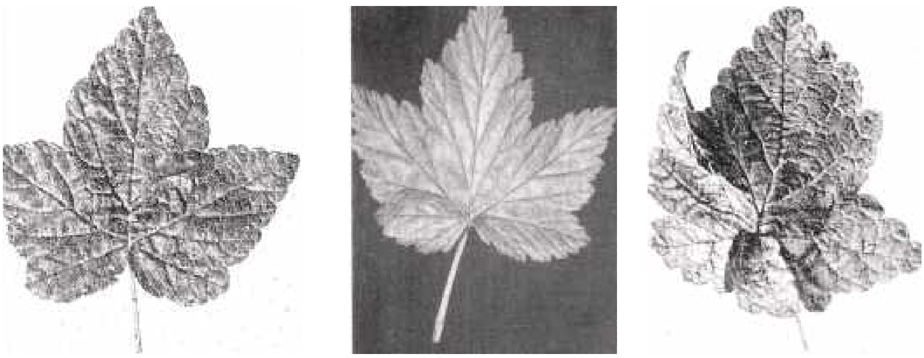
However, the doubling of the gooseberry genome leads to the disappearance of some features which are common to Ag. These features include the formation of two gemmae instead of one in the leaf base, the umbrella shape of the raceme and pluripartite leaves. It can be assumed that the appearance of these features is associated with such phenomenon as fasciation. The disappearance of fasciations in Td is the proof that they are not derived from the original forms, but they are the result of interaction of parental chromosomes, which are equal in number.

Adding gooseberry genome in BGGG allotetraploid increases the expression of its features even more. This affects the color and the nature of the shoots, the color of the gemmae and their position on the shoot, the shape and the color of the leaves, petals and sepals, the form of ovary, stile, fruit, fruit color and the peel type, and the hybrid inability to reproduce with woody sprigs.

The increase in the number of chromosomes of a black currant from 8 to 16 leads to the strengthening of its characteristics in hybrids with the genomic composition of BBG. This is manifested in the color of shoots and the nature of their surface. Td BBG have vinous and exfoliating shoots. The strengthening of black currant properties is also expressed in following: the predominance of 5-lamina leaves, dark green color, the absence of burrs on the shoots, crinkled nature of the upper side of the leaf, pubescence deficiency on the lower side of the leaf, the occurrence of white rare tips on serratures of the leaf edge (they are absent in Td BGG), the prevalence of 5-7-flowered brush (Td BGG have dominated solitary flowers, rarely 2-flowered brush), pubescence flower raceme (Td BGG have naked racemes), and the position of the sepals. In the presence of two blackcurrant genomes, the following characteristics as the shape and color of the berries, matte fruit peel and such undesirable characteristics as misaligned fruits in a raceme are steadily dominated. Regardless of the number of the genomes, the *Ribes* attribute as stames location at the same level with petals and *Grossularia* attribute as sprawling type of the bush, deficiency of ethereal glands and the ribbed ovary are steadily dominated.

From the above comparison, it is seen that in amphidiploids, at the equal ratio of the number of chromosomes of initial species, *Ribes* homozygous condition of the genomes determines the dominance of many valuable features of currants. At the same time, such properties of the original forms as the amount of burrs on shoots and peculiar currant smell, despite homozygous condition of the genes determining their manifestation, are recessive.

Comparing the peculiarities of manifestation of the characteristics in Ad, Cd and Ag (Fig. 2), it may be noted that the number new structures develop only at equal ratio of the amount of genomes of original forms (1: 1 in Ag and 2 : 2 in Ad).



a b c

Fig. 2. The Leaves of *R. nigrum* x *Gr. reclinata*: a – amfihaploid (BG), b – allotriploid (BBG), c – amfidiploid (BBGG)

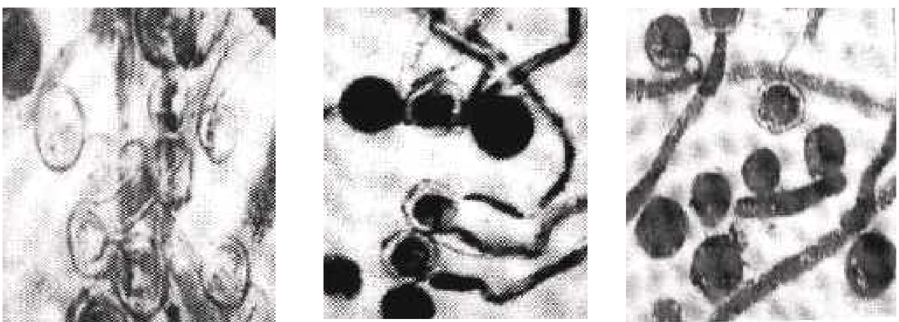
Source: own research

Changing the equal ratio of the number of genomes in Td such features as pluripartite shape of the leaves, the presence of two gemmae in the leaf base, the variation of the color of leaves, the length of the flower stem, and 5-7-flower raceme disappear. For expression of these features, not the dose of currant and gooseberry genomes is important but their equal ratio of 1: 1 or 2 : 2.

However, there are a number of features, which are manifested only at the ratio of a certain number of chromosomes of original forms. These features appear only at the ratio of 2 : 2 and disappear at 1: 1. Here, first of all, Ad fertility and Ag sterility are meant. In some cases, there has been an increase in currant characteristics with the addition of gooseberry genomes. For example, the position of the anthers in the flower can be explained by the change in the number of genomes which does not lead to a simple summation of the dose-response effect of each gene and causes allele interaction of the same and different genes at the genome increase, which can give an unexpected effect.

Comparing the character of the manifestation of the features with a doubling of the chromosomes of each parent, and analyzing amfihaploids (BG, 2n = 16) and amphidiploids (BBGG, 2n = 4x = 32) *R. nigrum* x *Gr. reclinata*, it is clear that at the doubling of the genomes of each parent, the character of the dominance of the properties in a number of cases varies. So, Ad compared to Ag, the enhancement of some currant properties is observed. The character of the surface of the shoots is changed. They are smooth and grey-brown, while the Ag shoots are gray-maroon and exfoliate. Ad gemmae have rounded shape with a blunt tip that makes them similar to currant gemmae, while Ag gemmae are conical, pointed, with tightly adjoined squame. With black currant Ad, unlike Ag, they have similarities in the absence of pubescence on the lower side of the leaf, in the nature of the base form of the leaf, and in the leaf edge with scanty white tips. In Ad the expression of currant characteristics in color and shape of the berries is enhanced. Therefore, the presence of a single currant genome suppresses the mentioned properties, as apparently *Ribes* genes controlling them are recessive. In doubling the number of *Ribes* chromosomes, the phenomenon of the dominance of these properties is more explicit despite the double number of *Grossularia* genomes. At the same time, some properties of *Grossularia*, which were recessive in amphihaploids, dominate in amphidiploids. They include the dense arrangement of gemmae square, large ovary, the form of the fruit flattened at the poles, almost smoothed the size of the fruit, and dark-maroon color of berries. Consequently, for the domination of a number of valuable features of *Ribes* and *Grossularia*, it is necessary that the genes of these species are in the homozygous state.

Certain properties don't change the nature of domination at doubling the genomes of parental forms. In amphihaploids as well as in amphidiploids the following properties of *Ribes* dominate: the shoots are without burrs, shrunken character of the upper side of the lamina, predominance of the 4-6 flower raceme and its pubescence, sepal color, the form of the hypanthium, anther position, and good rooting ability of the sprigs. The dominant properties of Ag and Ad of *Grossularia* have the deficiency of aromatic glands, the ribbed surface of ovary, the duality and pubescence of the stile. The hybrids with 2n = 16 and 2n = 32 have the following dominant agricultural features: high winter tolerance, immunity and resistance to currant gall mite. Amphihaploids are fully sterile. They bloom heavily, setting single seedless fruit. Amphidiploids are normally fertile and differ from amphihaploid plants in the level of pollen fertility (Fig. 3).



a b c

Fig. 3. Pollen of *Ribes nigrum x Gr. reclinata*: a – amfihaploid (BG), b – allotriploid (BBG), c – amfidiploid (BBGG)

Source: own research

Our research has shown that amfidiploid forms have morphologically balanced pollen (from 79,8 ± 1,04 to 86,4 ± 2,11% in different years), normal size (49,6 ± 1,16-50,1 ± 0 44 microns), and a high percentage of germination (from 60,6 ± 0,16 to 69,6 ± 0,84%). Morphologically normal pollen in amfihaploids consists of only 0,96 ± 0,09-1,32 ± 0,01%. Pollen grains are small (20,1 ± 0,18-22,8 ± 30 microns). They do not germinate on artificial nutrient medium.

26,8 ± 0,06-54,0 ± 0,35% berries are set in Ad in open pollination, but Ag only have single seedless fruits. Ad berries are large (1,02 ± 0,14-1,2 ± 0,17 g), oval, slightly flattened at the poles, of dark vinous color, with a smooth shiny peel, and of sweet-sour taste. The berries in size are almost flattened and ripen in mid-August. The number of seeds in a berry is approximately from3,4 ± 0,19 to 5,6 ± 0,63. The average weight of 100 seeds is equal to 0,36 ± 0,01-0,44 ± 0,02. The seed plumpness is not less than 76.2-81.3%. Seed germination is from 30,6 ± 1,02 to 38,0 ± 0,05%. Seedlings don't appear synchronically during 20-32 days. Single Ag berries are small with the weight of 0,19 ± 0,03 g. Seeds do not develop in them.

The formation of fertile pollen and viable seeds give evidence of the normalization of the meiosis process during sporogenesis when compared to Ag sterile forms. Ad fertility allows using them in crosses with gooseberries and currants to enhance the desirable traits. Uncrossing with diploid forms of *Ribes* and *Grossularia*, Ad interbreeds with their tetraploid forms. When pollinating of autotetraploid form of *Gr. reclinata* (BBGG genomic composition) with the pollen of amphidiploids *R. nigrum* x *Gr. reclinata* (genomic structure is BBGG), allotetraploids with new genomic composition BGGG were obtained, but after pollination of autotetraploid *R. nigrum* (genomic structure BBBB) with the pollen Ad, allototetraploids with the genomic composition GBBB were gained.

CONCLUSIONS

1. The obtained reciprocal amfihaploids of *R. nigrum* x *Gr. reclinata* with genomic composition such as BG and GB (2n = 16) differ from the original parental forms and hybrids with another genomic composition by the nature of the growth and shoots colour, the adjacency of bud squames, the bud forms, and also by the size of leaves, flowers, and flowers in the racemes.
2. The sustainable sterility doesn’t allow to use them directly for practical purposes. However, a number of valuable new structures, typical for amphihaploids, gives the possibility to consider them as a valuable source of breeding material for further breeding.
3. The reciprocal amphihaploid forms of *R. nigrum* x *Gr. reclinata* with genomic composition such as BGG and GBB (3n = 24) can be used as an intermediate link in the preparation of allotetraploids with the genomic composition such as BGGG GBBB, as well as diploid fertile recombinants with economically valuable traits.
4. Amphidiploids of *R. nigrum* x *Gr. reclinata*, with the genomic composition of BBGG and GGBB (4n = 32), are characterized by a complex immune system, high winter tolerance, a high percentage of normally formed pollen grains, large-sized fruits, and a small quantity of seeds.

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SUMMARY

It was established that amfigaploidy differ from the original parental forms with character of growth and color shoots, close fitting bud palet, bud shape, the size of leaves, inflorescence, flowers in the flower racemes. It was determined the possibility of using allotriploidny forms as an intermediate in the preparation of allotetraploid and fertile diploid recombinants with economically valuable traits. It was identified that some forms of amphidiploids have the complex shapes immunity, increased winter hardiness, fruits with large and small seeds.