Accounting unit in hybridological analysis

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Abstract. The world is dedicated to studies recombinogenesis in evolution and selection, on the variability causes of recombination and segregation parameters in hybridological analysis. The necessity of a careful choice in control unit for recombination experiment dependent on the architecture of a species reproductive system.

1 Introduction

In the field of interests of ecological genetics of cultivated plants, we have studied the issues of control of the morphogenetic process during hybridization: the causes of recombination variability and methods of its assessment in higher plants at the organ, organism and population levels; the role of the architectural features of the reproductive systems of a species in recombination processes in time and space; the influence of the sectoral transport of assimilates on the formation of recombinant gametes and zygotes in anthers, flowers, fruits, inflorescences, etc. in the system of recognizing "friend or foe" with artificial regulation of reproductive load; dependence of the synchronicity of reproductive and recombination processes in time and space and their influence on the processes of morphogenesis; the relationship between the level of recombination and the degree of selective elimination of gametes and zygotes in different exogenous conditions; the dependence of the recombination frequency on the magnitude of heterosis and the competitiveness of hybrids; the effect of the crossing over frequency on quasi-entanglement, etc. [1, 2, 3, 4]. The relevance of these issues is due to the versatility of various mechanisms for regulating the form-forming process in a plant at different stages of the breeding process with a comprehensive consideration of the problem of “plant as a system”.

For example, the difference in the release of the spectrum of genotypic variability is of particular importance in higher organisms, which can be due to a short or relatively long life cycle of development. It is obvious that the genetic systems that control the yield of genotypic variability in phylogeny and the genetic systems that control ontogenesis should not be selectively neutral; the influence of reproductive systems on the formation of potential and free recombination variability was revealed. Thus, self-pollinating species of higher plants, as a rule, are characterized by a high frequency of chiasmata and the release of relatively significant genotypic variability; on the contrary, in cross-pollinated plants, the frequency of

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exchanges for bivalent is usually lower and the release of variability, as a rule, occurs regularly from generation to generation, but in small portions [4], which explains one of the compensatory mechanisms in the formation of recombination variability in higher plants, providing a balanced realization of the potential of genotypic variability in ontogeny and phylogeny, in particular, depending on the breeding system”.

2 Materials and methods

Experiments are presented on tomato and flax. Controlled crosses and identification of marker genotypes in splitting populations were carried out. Additionally, cytological studies of the formation of chiasmata in meiosis were carried out.

3 Results and Discussions

Many researchers have established the existence of significant recombination variability at the organ, organizational and population levels. Thus, for a number of objects of higher plants, such as corn, tomato, horse beans, rye, peas, Arabidopsis, etc., a significant age-related dynamics in the frequency of recombinations has been established [1, 4, 5]. In higher plants, the frequency of crossing over in a marked segment can vary between inflorescences of one plant, and with an increase in the order of arrangement of the inflorescence on the stem, the frequency of crossing over decreases significantly. Perhaps this is due to age, evolutionary specific features in the nature of the distribution of meiotic events within a plant (between inflorescences) and even individual reproductive structures (hand, ear, etc.), depending on exogenous and endogenous environmental conditions constantly changing over time. For example, the variability of the values of the crossing over frequency on the first and subsequent inflorescences in tomato can be explained not only by the different influence of environmental factors on meiosis and postmeiosis, but also by the peculiarities of the ontogenetic “memory” of each inflorescence and even flower, selective elimination of recombinant gametes and zygotes, selective elimination of recombinants with shedding of certain flowers and underdeveloped fruits, a decrease in the elimination of recombinants under stressful conditions due to a decrease in competition between reproductive organs for a limited nutrient substrate with artificial regulation of reproductive load [4]. Ontogenetic “memory” of the components of genetic programs of ontogenetic and phylogenetic adaptation can act for fast-flowering or long-flowering higher plants as a mechanism providing the distribution of protection when the potential of genotypic variability is realized, depending on changes in environmental conditions (exogenous and endogenous) during the reproductive development of plants and associated with the duration stages of meiosis [4].

As a rule, in the overwhelming majority of higher plants, flowers are not located singly, but form groups with a certain spatial arrangement and a sequential order of their initiation. In many cases, flowers in inflorescences (brush, spike, head, scutellum, umbrella, curl, gyrus, etc.) and inflorescences on plants, pollen and ovules in buds are laid one after another with small time intervals during the sector transport of assimilates in the section anthers, to ovules, flowers, seeds, fruits, inflorescences, etc., with a certain temporal and spatial passage of the stages of meiosis and fertilization in critical (unprotected from stress) periods of reproductive development [4]. Many researchers consider the reproductive development of higher plants to be the decisive stage in ontogenetic and phylogenetic adaptation, and the development of each reproductive organ is one of the unique “reproductive episodes”. For example, the plant reproduction system can act as a kind of receptors for the action of environmental factors, the ecological "memory" of which is manifested in the change of sexual reproduction to vegetative reproduction, strengthening or weakening of inbreeding or outcrossing, etc.,
which ultimately can contribute to different formation of recombination variability due to changes in the frequency of recombinations and/or the degree of elimination of recombinant gametes and zygotes [2, 4, 7].

Mechanisms are known that lead to the differentiation of reproductive organs in the ontogeny of higher plants. Thus, numerous studies confirm the concept of internal (endogenous) competition for nutrients as the main factor of apical dominance and associated subsequent processes of autonomy of reproductive organs within a plant, inflorescence, or an individual reproductive organ. Mechanisms such as genetic control of the development of reproductive structures and ontogenetic instability of gene expression, influence on the reproductive phase of cytoplasmic heredity and the phenomenon of heterosis (including "fading heterosis"), the relationship between recombination and heterosis, the level of crossing over and quasi-linkage frequency, age-related variability and the peculiarities of selective fertilization and zygotes in connection with the architecture of the reproductive system of the species, selective elimination of fruits and the role of attracting centers, donor-acceptor relationships and sectoral transport of assimilates, the effect of the reproductive load on the development of reproductive organs and structures, etc. All this indicates a set of evolutionarily established mechanisms that provide flexibility and plasticity of the reproductive apparatus of angiosperms, contributing to a specific response to changing conditions of existence, as well as distributing the formation of recombination variability in time and space, which ultimately contributes to a more complete understanding of the mechanisms of regulation of the morphogenetic process in higher plants and the release of potential genotypic variability [4, 7].

The dynamism of the formation of recombination variability at the organismal level is significantly enhanced by a set of mechanisms that determine the release of potential genotypic variability at the level of the reproductive organ (organ variability). Thus, in higher plants, in particular for primrose, corn, peas, tomato, onion, pine, Arabidopsis [1, 2, 4], etc., significant discrepancies in the values of the frequency of recombinations in micro- and macrosporogenesis have been established. In the studies of most authors for various objects, it was shown that the frequency of crossing over in microsporogenesis is sometimes higher than in macrosporogenesis. It is assumed that sex restrictions on the frequency of recombinations can contribute to "equilibrium of linkage and balance" for different loci in the presence of selective elimination of gametes and zygotes, or selective pressure in favor of enhancing the linkage of genetic factors that determine the main differences between the sexes. There are also experimental data on organ recombination variability in different parts of the anthers of rye, tulip, and tomato [4, 7]. For example, in the anther of tomato plants, there is a significant chiasm frequency gradient from the base to its free end: the cells that first entered meiosis at the base of the anther have the lowest frequency of chiasm, and the cells that entered meiosis last at the free end of the anther are characterized by the highest frequency chiasm [1]. Of practical interest is the consistency of the sequence of meiotic divisions and the differentiation of nutrient intake in the anther section with a certain distribution of the level of chiasm frequency in such relatively different objects as rye and tomato. Therefore, it can be assumed that organ variability in the frequency of recombination in micro- and macrosporogenesis, as well as in different sections of anthers, may have an adaptive value, leading to differences in the level and spectrum of released genotypic variability in successive groups of offspring of the same individuals within the same reproductive organ. At the same time, one of the main mechanisms of the formation of recombination variability at the organic level for most higher plants is probably a selective feature at the postmeiotic stages of recombinant gametes and zygotes. For example, in tomato and maize, selective elimination of recombinant gametes and zygotes was established, which more often occurs at the stages of gametogenesis, pollen germination and pollen tube growth, fertilization, embryo development, and seed formation and germination [1, 4].
It is known that the vast majority of flowering plant species have enough pollen to fertilize all ovules. However, self-pollinators, relative to cross-pollinated species, are usually characterized by a lower ratio of pollen grains and ovules in one flower (3). At the same time, the genetic diversity of pollen can contribute to different competitiveness of microgametes (mainly due to differences in the growth rate of pollen tubes in the tissues of the pistil), which, in turn, will determine the nature of the distribution of the genotypic and modification heterogeneity of zygotes and seeds within the same reproductive organ. The fitness of the whole plant and the specificity of the metabolism of an individual flower can significantly affect the direction and level of pressure of internal selection, the elimination of recombinant gametes and zygotes, which is associated with the specific architectural features of the reproductive systems [4, 5, 7].

Thus, the connection between the recombination system and the reproductive system in higher plants, due to the evolutionary set of mechanisms that contribute to the formation of recombination variability at different levels (population, organismal and organ), provides a balanced realization of the potential of genotypic variability. The adaptability of this connection is manifested in the evolutionarily determined species specificity of the architecture of the reproductive system in the nature of the sequential development of the reproductive organs and structures on the plant in time and space; the number of gametes, zygotes and viable offspring of one reproductive organ, inflorescence and plant; the characteristics of the reproduction system and the duration of fertilization, meiosis, postmeiosis, life cycle, etc. The level and spectrum of recombination variability depends not only on the specific environmental conditions prevailing during meiosis and postmeiosis, but also, possibly, on the characteristics of the ontogenetic "memory" of the reproductive system in general, each inflorescence, flower and even anther. The balanced effect of a set of endogenous and exogenous factors on recombigenesis at different levels is manifested in the relatively regular formation of new genetic variants in the reproductive cycle, which contributes to the periodic testing of new gene combinations and the protection of old coadapted gene blocks. As a result, higher plants create a balance between ontogenetic and phylogenetic adaptability, stability and variability, between “present” and “future” in terms of the criteria of natural evolution. This implies an applied nature in the development of principles and methods for managing the morphogenetic process, that is, shifting the indicated balance in favor of the “present” based on knowledge of the peculiarities of the formation of recombination variability in higher plants at different levels: organ, organismal and population [1, 8-12].

There is a problem of choosing a "unit of account" in hybridological analysis, which is, perhaps, closely related to the issue of assessing the reliability of an established fact, that is, with determining the likelihood of how random or logical the result is. For example, the question arises as to what the optimal or minimum number of individuals in F2 should be in order to determine the significance of the deviations of the cleavages from the theoretically expected ratio of 3:1, which obviously depends on the biological features of the architecture of the reproductive system of a species, variety or form (the nature of reproductive development - the duration of meiosis and/or the number of pollen grains and ovules in the flower of the offspring from one fruit, inflorescence, plant, etc.). At best, such a unit of account should meet the following criteria: recombination in meiotic cells of a heterozygote, as well as postmeiotic processes, should take place under relatively identical conditions. This issue requires special consideration on the basis of special experiments and in-depth study of the numerous experimental data available in the literature for various objects with different features of recombination and reproductive systems. On the other hand, the use of a fruit or other structural component of the reproductive system as a unit of assessment and selection in selective practice is largely due to the experimentally established patterns of distribution of genetic variability within a plant, depending on the growing conditions of a particular
crossing combination, and has not been sufficiently studied for many cultivated species. At the same time, our experiments and published experimental data of various authors indicate that in tomato the frequency of recombinations in individual zones of the genome is significantly dependent on the order of inflorescence on the main stem of the plant. The first inflorescence is more often characterized by high values of crossing over frequency relative to the second, third and subsequent inflorescences. The observed differences in the frequency of recombination can contribute to a different degree of selective elimination of recombinants between inflorescences and individual buds within the same F1 inflorescence, due to the conditions prevailing under the influence of biotic and abiotic factors when the reproductive organs pass through the corresponding stages of development - from the establishment of generative structures to seed formation. In this case, the fruit of tomato F1 hybrids plants can act as the main "cell" in the realization of potential and selectable genotypic variability.

It is known that recombination variability and the degree of elimination of recombinants at postmeiotic stages may depend on heterozygosity, heterosis and competitiveness of F1 (in terms of productivity), the age of the heterozygous organism, the conditions of its cultivation, etc. the breeding process of factors that induce recombination variability, such as soil drought, deficiency of mineral nutrition, air temperature, thickening of F1 hybrids under environmental stress, etc. Along with the influence of abiotic factors, it is important to isolate the role of genetic factors in the formation of recombination variability at different levels (population, organismic and organ).

Within the framework of the problem under consideration, hybrid heterozygosity deserves special attention, which plays the main role in the release of free and available genotypic variability. In this regard, the question arises whether the potential of genotypic variability available to the breeder in F2 increases with an increase in the level of heterozygosity in F1, and how this is related to the nature of the adaptability of hybrid combinations, reproductive load, the influence of different ontogenic adaptability of genotypes in terms of the degree of competitiveness, taking into account recombination variability. At the same time, how to use competition in the breeding process as a recombinogenic factor or background for selection, or both, is still insufficiently studied, although work in this direction is being carried out quite intensively [4, 3-15].

Obviously, the nature of the manifestation of the diversity of offspring to a large extent depends on the stabilizing natural selection, which can significantly limit the artificially generated free variability, eliminating the most valuable genetic combinations. The action of natural selection at all stages of the reproductive cycle of a heterozygous organism and postmeiotic processes is due to a variety of both endogenous and exogenous factors. We have studied the factor of “limited” reproductive load under stress conditions, which meets the requirements of a systemic approach, since it can affect "all (and even so far inaccessible to artificial selection) stages of postmeiotic elimination and is probably important in studying its effect on the nature of selective elimination recombinant gametes and zygotes. In this regard, the experimental data available in the literature are still focused on studying the effect of an artificially limited reproductive load on metabolism, a decrease in competition between reproductive organs for a limited nutrient substrate, etc., of cultivated higher plants, especially under stress conditions [4]. However, along with the widespread use of physical, chemical recombinogenic agents in order to increase genotypic variability, powerful evolutionary factors and structures of species, such as heterozygosity, competition, heterosis, architecture of the reproductive species, etc., were not sufficiently involved in the development of methods that improve breeding process.
4 Conclusions

Thus, extensive experimental data on the variability of recombination characteristics under the influence of environmental factors, the dependence of objective assessments and practical results on a differentiated approach to a plant as a system, taking into account the biological potential of higher plant species, can contribute to the cognition of a set of mechanisms that determine the formation of recombination variability at different levels, which will allow to supplement the methodology for its assessment, as well as ensure the creation of new and effective genetic methods that intensify the breeding process.

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