Theoretical and Applied Genetics 41, 259–262 (1971)

Methods for Induction and Utilization of Variability in the Improvement of an Apomictic Grass, *Dichanthium annulatum* Complex

A. P. SINGH and K. L. MEHRA

Division of Plant Improvement, Indian Grassland & Fodder Research Institute, Jhansi, U.P. (India)

Summary. Many problems and difficulties are encountered in making genetic improvements in plants where both apomixis and polyploidy occur together. From biosystematic studies on an agamic species complex, *Dichanthium annulatum*, information is presented on: (A) Mechanisms which create variability in apomicts — (i) genome building and reduction, (ii) hybridization between ecotypes of facultative apomicts, (iii) fertilization of unreduced gametes, (iv) introgressive hybridization, (v) preferential pairing and genotypic control of bivalent formation and (vi) induced mutation; (B) Embryo-sac variations, vis-a-vis sexual/apomictic sacs — (i) production of sexual embryo-sac in apomicts, (ii) balance between apomixis and sexual process, (iii) effect of environment and experimental manipulation of the type of embryo-sac; and (C) Heterosis and fixation of apomixis.

The utilization and exploitation of these mechanisms and phenomena for accelerating the genetic improvement of apomictic plants is discussed.

Mating systems impose certain restrictions on the breeding methodology to be used in the genetic improvement of crop plants. Allogamous species have built-in mechanisms for self-improvement and, for them, the breeding techniques are well worked out. Little information is, however, available on the procedures to be followed for the genetic improvement of apomicts. Recently gathered information on the causal mechanisms of apomixis and its mode of inheritance, the genetic systems which regulate the balance between apomixis and sexuality, the physical and chemical agents for artificial induction of sexuality in apomicts, and the processes which promote variability and adaptive polymorphism in apomicts show a way for the creation, exploitation and fixation of superior genotypes. Such information, based on biosystematic studies on an agamic species complex, *Dichanthium annulatum*, at the Oklahoma State University, Stillwater, Oklahoma, U.S.A., is presented here.

Breeding procedures commonly followed for the genetic improvement of apomicts are outlined below:

1. Collection of varieties, strains or ecotypes from diverse sources; 2. Evaluation of the germ plasm for the presence of desirable characters; 3. Building up of selection indices and estimation of genetic parameters; 4. Determination of mode of reproduction and isolation of sexual types or clones; 5. Hybridization using the sexual types; 6. Progeny testing, comparisons, multiplication and release of superior types.

Thus, the success of the breeding programme would depend on the range of variability already present in the germ plasm collections, the relative proportion of sexual/apomictic seed produced and the exploitation of variability from the crossbred progenies. Since large collections of plants with different genotypes are not often available, one would like to look for the mechanisms which can create variability in the apomicts. Such mechanisms are as follows.

A. Mechanisms promoting variability in apomicts

1. Genome-building and reduction

Chromosome races are produced through the doubling of chromosome numbers of sexual diploids in diploid × tetraploid crosses, the fertilization of cytologically unreduced eggs with reduced gametes in tetraploid × tetraploid crosses, hybridization between plants with different ploidy levels and the parthenogenetic development of cytologically reduced eggs of the polyploids (Singh and Mehra, 1965).

2. Hybridization between ecotypes of facultative apomicts

Four distinct ecotypes are recognized within the *D. annulatum* complex (Celarier *et al.*, 1958; Mehra, 1962) and both artificial (Singh *et al.*, 1962; Singh and Mehra, 1965; Singh, 1968) and natural hybrids (Mehra, 1966) are reported between them. In this agamic complex, the tetraploids are facultative apomicts, the hexaploids are obligate apomicts and the diploids are always sexual (Celarier and Harlan, 1957). Hybrids between the facultative apomicts were either apomictic or sexual (de Wet and Borgaonkar, 1963). Wide genetic recombination and transgressive segregation were observed in the F_2 progenies of both tetraploid and hexaploid F_1 hybrids between $4n$ tropical and mediterranean ecotypes of the *D. annulatum* complex (Borgaonkar and Singh, 1962; Mehra, 1964 a).

3. Fertilization of unreduced gametes

Hexaploids ($2n = 60$) were often produced by fertilization of the unreduced egg with the reduced gamete in $4n \times 4n$ crosses. This mechanism permits the incorporation in the hybrid of an entire chromosome complement of the female parent and half of the chromosome complement of the male parent. In fact, *D. papillosum* (Hochst.) Stapf seems to have originated from the fertilization of an unreduced egg of the tetraploid tropical ecotype with the reduced gamete of the tetraploid mediterranean ecotype of *D. annulatum* (Forssk.) Stapf (Borgaonkar and Singh, 1962; Mehra, 1964 b).

4. *Introgressive hybridization*

Introgression occurs between: (i) the tropical and mediterranean ecotypes of the *D. annulatum* complex from Afghanistan to North-West Africa (Mehra and Celarier, 1958; Mehra, 1962); (ii) *D. annulatum* and *D. fecundum* S. T. Blake in Australia (Borgaonkar and de Wet, 1964); and (iii) *D. annulatum* and *D. papillosum* in North-East Africa (Mehra, 1966). Natural hybrids between *D. annulatum* and *D. caricosum* (L.) A Camus, and between the latter and *D. aristatum* (Poir) C.E. Hubbard are also reported (Celarier *et al.*, 1961). Furthermore, *D. annulatum* introgresses with *B. intermedia* in the Gangetic Plain of India (Harlan *et al.*, 1958). Also, natural hybrids between *B. intermedia* and *D. annulatum* introgress with *B. ischaemum* (L.) Keng in North-West Pakistan (Harlan, 1963). Thus, as a result of introgressive hybridization involving the ecotypes of the *D. annulatum* complex and the related species, wide variability is produced.

5. *Preferential pairing and genotypic control of bivalent formation*

Meiotic irregularities are common in most of the apomictic species, especially those associated with high polyploidy. However, cytological evidence has indicated that in the *Bothriochloa-Dichanthium* agamic complex compulsive bivalent pairing was controlled by a single dominant gene (Chheda and Harlan, 1962). Also, the chromosome homology, homoeology and non-homology is a matter of degree due to the level of introgression from *Bothriochloa* species into the genomes of *D. annulatum* ecotypes (Mehra and Singh, 1968). Such a situation would eventually tend to break down the genomic relationships, reduce multivalent formation, cause genetic segregation and recombination of new genotypes, leading to a further release of variability.

6. *Mutation*

In obligate apomicts, expressing the lowest sexuality, mutation provides the main source of variability in the population. Bisexuality of the pedicellate spikelets in *D. fecundum*, a character governed by a single gene (Borgaonkar and de Wet, 1960), is an example of such a process. Variability could also be introduced through the use of mutagenic agents.

It must be emphasized that facultative apomixis, coupled with the above mechanisms promoting variability, will, by its combination with the sexual and apomictic embryo-sac, achieve a compromise similar in principle, and probably in effect, to that brought about by combining the advantages of the sexual as well as asexual reproduction. Consequently, experimental manipulation and exploitation of embryo-sac variations, vis-a-vis sexual/apomictic sacs, is likely to open up further avenues for accelerating the genetic improvement of apomicts.

B. Embryo-sac variations, vis-a-vis sexual/apomictic sacs

1. *Production of sexual embryo-sacs in apomicts*

In facultative apomicts, both sexual and asexual embryo-sacs may occur concurrently. The apomictic fraction is usually more fertile, vigorous and enjoys a selective advantage. In *Dichanthium* and its related genus *Bothriochloa*, sexual sacs are produced even in obligate apomicts (Brooks, 1958). Most apomictic collections of *D. annulatum* differ in the relative frequencies of the sexual and asexual embryo-sacs, the latter being more numerous than the former (Brooks, 1958).

2. *Balance between apomixis and sexual process*

The potential to develop the sexual embryo-sac is always present in the facultative apomicts. Consequently, depending upon the genotypes of the parents and the relative frequency, fertility and vigour of the apomictic and sexual fractions, the hybrids may reproduce either apomictically or sexually. Apomixis in the *Bothriochloa-Dichanthium* group appears to be controlled by two genes at the tetraploid level and apomixis is dominant over sexuality (Borgaonkar *et al.*, 1962; de Wet and Borgaonkar, 1963). The situation here seems to differ from that in *Potentilla* in which it is not only the individual genes but also the relative proportion of the genomes from the sexual and apomictic parents which determines the type of seed produced (Müntzing, 1958). In this context, the genetic model presented by Powers (1945) in *Parthenium argentatum* deserves careful consideration. Thus, once the cytogenetical evidence is available to locate the position of gene or gene complexes responsible for such mechanisms, the segment of a chromosome contributing to it could be incorporated in the genotype of a desirable plant by a translocation technique like that accomplished in wheat (Sears, 1956). Such manipulation is likely to have a tremendous impact on the future breeding methodology of apomicts.

3. *Effect of environment on type of embryo-sac*

Photo and thermo-periods are the important components of the environment regulating the embryo-sac development (Heslop-Harrison, 1961). In *D. aristatum*, a short-day plant, length of day during the development of the inflorescence is strongly correlated with the incidence of apomixis, and pollen fertility also varies according to the photoperiodic conditions (Knox and Heslop-Harrison, 1963, 1966 and Knox, 1967). Thus, when the day-length in *D. aristatum* exceeds 14 hrs. throughout the development of the inflorescence, the incidence of apomictic sac is low compared to that when day-length is less than 14 hours. Similarly in *D. annulatum*, the relative frequencies of the sexual and aposporic embryo-sacs fluctuate with the growing season (Gupta *et al.*, 1969). Thus, by regulating the environmental conditions conducive to the type of embryo-sac development in the facultative apomicts, the breeder could induce the plants to produce the sexual sacs when hybrids are to be obtained, or the apomictic sacs, when the maternal seeds are to be obtained.

4. Experimental manipulation of type of embryo-sac

Induction of sexuality in the apomicts and vice-versa by the use of physical or chemical agents is one recent approach to shifting the balance between apomixis and sexuality. Asker (1966) was able to increase considerably the number of sexual plants in the facultative apomicts of *Potentilla* by the use of X-ray. The encouraging results obtained in *Potentilla* have opened the way to making a concentrated effort on those apomicts which are potentially most responsive to such mutagenic agents. Another advantage of mutation breeding is that when the useful variation latent in many apomictic species is released by these or other means, the apomictic mode of reproduction can greatly facilitate the subsequent selection, maintenance and release of the new varieties for commercial use.

C. Heterosis and fixation of apomixis

The use of clones capable of producing a higher proportion of sexual than apomictic sacs as parents in desirable crosses, with the mechanisms leading to a further release of variability outlined here, could secure wide variability for exploitation in the breeding programme. The superior genotypes thus obtained in the progeny might already be apomictic, or apomixis could be incorporated in them. Consequently, the heterotic vigour, once obtained, could be stabilized through apomixis. In the case of distant crosses, where balanced gametes are not produced in the F_1 plants because of meiotic irregularities, apomixis provides a mechanism to overcome such barriers to seed production.

We can conclude that, contrary to the previous belief that apomixis is a blind alley in evolution, genetic improvement is possible in apomicts, especially where it is of a facultative nature, and the balance with sexual reproduction is both genetically and environmentally controlled. Though some progress has been made, many problems concerning the genetic basis of apomictic seed formation are still unsolved. By and large, the facultative apomictic mode of reproduction is of advantage to the breeder in that by taking advantage of the mechanisms promoting variability and by fixing the heterotic genotypes through apomixis, not only can he exploit hybrid vigour but he can also maintain uniform populations of such genotypes.

Acknowledgement

The authors wish to express their sincere thanks and sense of appreciation to Shri P. M. Dabadghao, Director, In-Charge, Indian Grassland and Fodder Research Institute, Jhansi, for his encouragement.

Bibliography

1. Asker, S.: Effects of mutagen treatment on some apomictic *Potentilla* species. Hereditas **55**, 249—265 (1966). — 2. Borgaonkar, D. S., Harlan, J. R., de Wet, J. M. J.: Cytogenetical study of hybrids between *Dichanthium annulatum* and *D. fecundum*. II Proc. Okla. Acad. Sci. **42**, 13—16 (1962). — 3. Borgaonkar, D. S., Singh, A. P.: Species relationship in *Dichanthium*. IV. *D. annulatum* and *D. papillosum*. Phyton (Argentina) **19**, 101—107 (1962). — 4. Borgaonkar, D. S., de Wet, J. M. J.: A cytogenetical study of hybrids between *Dichanthium annulatum* and *Dichanthium fecundum*. Phyton (Argentina) **13**, 137—144 (1960). — 5. Borgaonkar, D. S., de Wet, J. M. J.: Cytogenetical study of hybrids between *Dichanthium annulatum* and *D. fecundum*. III. Phyton (Argentina) **21**, 55—66 (1964). — 6. Brooks, M. H.: A study of the reproductive mechanisms in certain species of the *Bothriochloa* and *Dichanthium* complexes. Ph. D. Thesis. Okla. State Univ., U.S.A. (1958). — 7. Celarier, R. P., Harlan, J. R.: Apomixis in *Bothriochloa*, *Dichanthium* and *Capillipedium*. Phytomorph. **7**, 93—102 (1957). — 8. Celarier, R. P., Mehra, K. L., Wulf, M. L.: Cytogeography of the *Dichanthium annulatum* complex. Brittonia **10**, 59—72 (1958). — 9. Celarier, R. P., de Wet, J. M. J., Richardson, W. L.: Species relationships in *Dichanthium*. I. Hybrids between *D. caricosum*, *D. aristatum* and *D. annulatum*. Phyton (Argentina) **16**, 63—67 (1961). — 10. Chheda, H. R., Harlan, J. R.: Mode of chromosome association in *Bothriochloa* hybrids. Caryologia **15**, 461—476 (1962). — 11. de Wet, J. M. J., Borgaonkar, D. S.: Aneuploidy and apomixis in *Bothriochloa* and *Dichanthium*. Bot. Gaz. **124**, 437—440 (1963). — 12. Gupta, P. K., Singh, A. P., Roy, R. P.: Aposporous apomixis, seasonal variation in tetraploid *Dichanthium annulatum* (Forssk.) Stapf. Acta Biol. **11**, 253—260 (1969). — 13. Harlan, J. R.: Natural introgression between *Bothriochloa ischaemum* and *B. intermedia*. Bot. Gaz. **124**, 294—300 (1963). — 14. Harlan, J. R., Celarier, R. P., Richardson, W. L., Brooks, M. H., Mehra, K. L.: Studies on the Old World bluestems. II. Okla. Agri. Exp. Sta. Tech. Bull. T-72, 1—23 (1958). — 15. Knox, B., Heslop-Harrison, J.: Experimental control of aposporous apomixis in a grass of the tribe Andropogoneae. Bot. Not. **116**, 127—141 (1963). — 6. Knox, B., Heslop-Harrison, J.: Control of pollen fertility through the agency of the light regime in the grass *Dichanthium aristatum*. Phyton (Australia) **11**, 256—267 (1966). — 17. Heslop-Harrison, J.: Apomixis, Environment and adaptation. In: Recent Adv. Bot. (Toronto Univ. Press) **1**, 891—895 (1961). — 18. Knox, R. B.: Apomixis: Seasonal and population difference in a grass. Science **157**, 325 to 326 (1967). — 19. Mehra, K. L., Celarier, R. P.: Cytotaxonomic notes on the *Dichanthium annulatum* complex. Proc. Okla. Acad. Sci. **38**, 22—25 (1958). — 20. Mehra,

K. L.: The *Dichanthium annulatum* complex. I. Morphology. Phyton (Argentina) **18**, 87—93 (1962). — 21. Mehra, K. L.: The *Dichanthium annulatum* complex. II. Relationships between the Tropical and Mediterranean types. Phyton (Argentina) **21**, 119—126 (1964a). — 22. Mehra, K. L.: The *Dichanthium annulatum* complex. III. Origin and artificial synthesis of *Dichanthium papillosum* Stapf. Caryologia **17**, 545—556 (1964b). — 23. Mehra, K. L.: The *Dichanthium annulatum* complex. IV. Study of natural hybridization from herbarium specimens. Rev. de Biol. **5** (3—4), 295—302 (1966). — 24. Mehra, K. L., Singh, A. P.: Chromosome manipulation and genotypic control of chromosome behaviour in *Dichanthium annulatum* complex and its hybrids. The Nucleus (suppl. vol.) **7**, 372—383 (1968). — 25. Müntzing, A.: The balance between sexual and apomictic reproduction in some hybrids of *Potentilla*. Hereditas **44**, 145—160 (1958). — 26. Powers, L.: Fertilization without reduction in guayule (*Parthenium argentatum* Gray) and a hypothesis as to the evolution of apomixis and polyploidy. Genetics **30**, 323 to 346 (1945). — 27. Sears, E. R.: The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symposium, 1—21 (1956). — 28. Singh, A. P., Harlan, J. R., de Wet, J. M. J.: Relationships within the *Dichanthium annulatum* complex. Proc. Okla. Acad. Sci. **42**, 50—54 (1962). — 29. Singh, A. P.: Hybridization range of *Dichanthium annulatum* (Forssk.) Stapf. Cytologia **34**, 563—574 (1968). — 30. Singh, A. P., Mehra, K. L.: Cytological analysis of hybrids within the *Dichanthium annulatum* complex. Cytologia **30**, 307—316 (1965).

Received December 1970

Communicated by H. Kihara

A. P. Singh, Botanist
K. L. Mehra, Head of Division of Plant Improvement
Indian Grassland and Fodder Research Institute
Jhansi, U.P. (India)