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PROSPECTS OF BREEDING IMPROVED GARLIC IN THE LIGHT OF ITS GENETIC AND BREEDING SYSTEMS

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SUMMARY

Garlic is an obligate apomict which defies all possibilities of genetic improvement through recombinant breeding. Nevertheless, like other bulbous or tuberous plants garlic has accumulated a lot of variation in many characters. Evidence of hybridity exists even at the chromosomal level. Existence of this natural variation, even in respect of the plant part that is economically important, suggests the possibility of improvement. The prospects of exploiting this variation in the face of apomixis are discussed.

INTRODUCTION

Of the ca. 600 species included in the genus *Allium* (STEARN, 1946), *A. cepa* (onion) and *A. sativum* (garlic) are the two most important representatives. Despite its world wide importance garlic has for some reason not drawn the attention of geneticists and plant breeders. As a result, even after centuries of cultivation, it exists more or less in the same form in which it was brought into domestication. The few changes that have been recorded, have by and large occurred spontaneously.

OBSERVATIONS AND DISCUSSION

Karyology

All clones so far studied have a diploid set of 16 chromosomes. This number is constant except for the sole case of $2n = 18$ reported by SHARMA & BAL (1958). A great deal of instability, however, exists at the structural level of chromosomes. BATTAGLIA (1963) has published an elaborate account of karyotype variation among the European clones. Although these clones exhibit significant differences in overall chromosome length, arm ratio, position of secondary constrictions etc., they are remarkably uniform in respect of the number of nucleolar chromosomes per haploid set. The few Indian cultivars studied hitherto are interesting because they exhibit a variation even in the number of nucleolar chromosomes (VERMA & MITTAL, 1978), similar to those observed by KÕNVICKA & LEVAN (1972). Somatic complements of a few clones are described below.

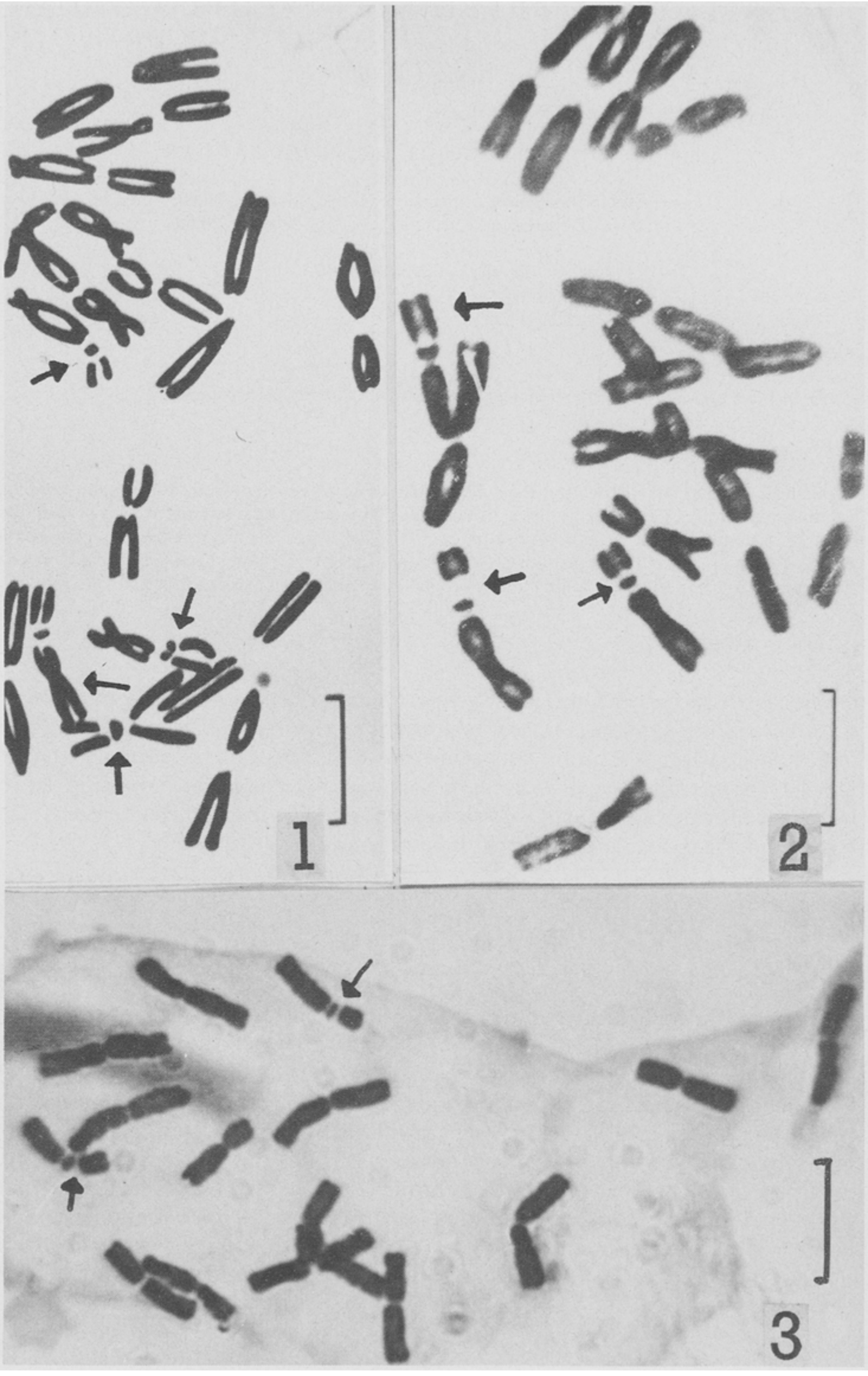
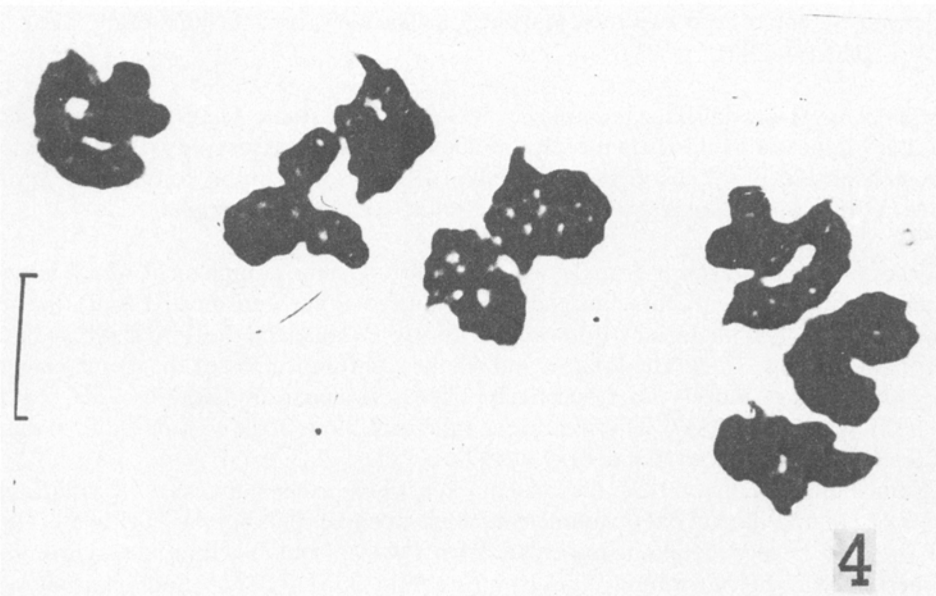


Fig. 1. A root tip cell from Dharwar clone showing 4 nucleolar chromosomes.
Fig. 2. Somatic complement of population collected from Srinagar, showing 3 nucleolar chromosomes.
Fig. 3. A root tip cell from clone of unknown locality bearing only 2 secondarily constricted chromosomes



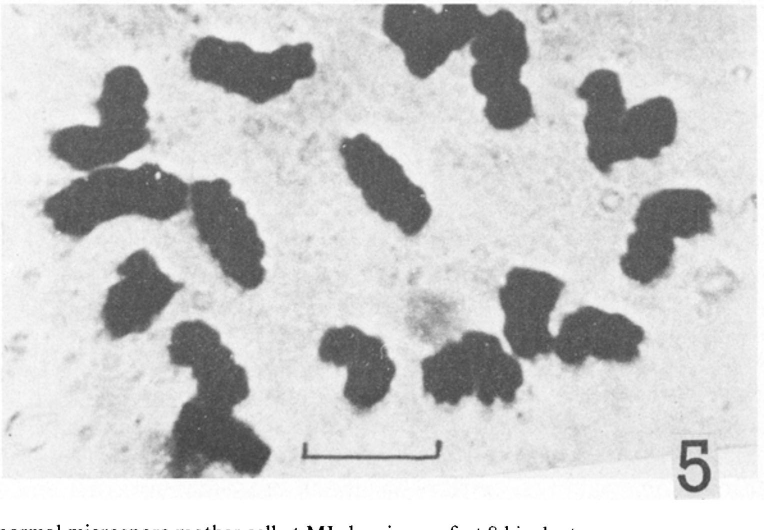


Fig. 4. A normal microspore mother cell at MI showing perfect 8 bivalents.
Fig. 5. A metaphase microspore mother cell from desynaptic clone showing 16 univalents.

Dharwar clone (Fig. 1) Bulb material was collected from Dharwar (Karnataka state). The karyotype consists of 2 median, 12 submedian and 2 subterminal chromosomes. The median pair occupies the first position in order of length. One pair from the submedian group and the subterminal pair of chromosomes bear secondary constrictions. All four secondary constrictions are of the 'sativum type' being located in the short arm. The markers occupy sixth and seventh positions in the idiogram. The longest and the shortest chromosomes of this karyotype measure 15.5 μm and 8.5 μm respectively, the ratio between these being 1.8. The karyotype formula of the clone is $2M + 10SM + 2SM^{sc} + 2ST^{sc}$.

Jaipur clone. It was collected in Jaipur in the State of Rajasthan. The essential details of the karyotype of this clone are the same as above. Differences exist only with respect to chromosome length. The longest and smallest chromosomes of this complement measure 18.7 μm and 9.2μm respectively, the ratio between the two being 2.

Garlics from the Kashmir Himalayas bear a chromosome complement which is peculiar in that it has only three instead of four nucleolar chromosomes (Fig. 2). In the Srinagar clone two nucleolar chromosomes are subterminal and the third is submedian (KOUL & GOHIL 1972). The longest and smallest chromosomes of this complement measure 15.5 μm and 9.4 μm, respectively. The karyotype formula of this clone is $4M + 9SM + 1SM^{sc} + 2ST^{sc}$. This somatic complement shows striking similarities to that of the UH clone of Kônvicka & LEVAN (1972).

Some bulbs purchased from the local market, whose exact source is not certain, are quite peculiar in having only two nucleolar chromosomes per diploid set (Fig. 3). The karyotype is interesting in one more character; the total lack of subterminal chromosomes. The karyotype formula of this clone is $2M + 12SM + 2SM^{sc}$. Such a karyotype has also been reported for a clone from Lund, Sweden, by KÔNVICKA & LEVAN (1972). Karyology of the Indian as well as European cultivars indicates the presence of a large genetic variation within this crop. This is, however, not of much consequence so long as it is not released through inbreeding and/or recombination breeding.

System of reproduction

In *A. sativum*, sexuality is thwarted at different stages of organogeny, sporogenesis and gametogenesis. Some clones do not produce exserted inflorescences at all. In those forming a scape, flowers are totally or partly substituted by bulbils with the result that the top sets are either perfectly vegetative or mixed. Development of flowers in some clones suggests that in the past garlic has had the means of sexuality, bulbifery representing a secondary change.

Studies on the male and female sporogenesis in some clones indicate that sporogenous tissue differentiates both in micro- and megasporangium. Within the female track, development ceases immediately after the egg mother cell undergoes reduction division but microsporogenesis proceeds uninterrupted. During pollen mother cell meiosis chromosome pairing is perfect and all cells bear eight bivalents (Fig. 4). Large numbers of chiasmata are formed and the average per cell varies from 24–42. Chromosome segregation at anaphase I is regular. Second nuclear division and cytokinesis proceed with precision and as a result microspore tetrads are formed. Development ceases at this stage and the microspores degenerate while still within the common wall; the flowers wither and drop off. In this way the sexual cycle breaks down half way without seed set (KOUL & GOHIL, 1972).

In a clone locally called 'Wan-ruhan' at Bhadarwah (Jammu and Kashmir) conditions of non-reduction and sexual sterility are imposed by desynapsis (Fig. 5). The 'ds' gene causes complete dissociation of chromosomes at the prophase itself with the result that the subsequent course of sporogenesis and gametogenesis is upset. This clone produces even pollen grains but they are largely non-viable.

KÔNVICKA & LEVAN (1972) reported amphibivalent formation in a clone of *A. sativum* collected from Sweden. This clone like others produced no viable pollen and therefore, turned out to be at least male sterile.

It follows from the above description that even such clones of garlic which bear flowers have the properties necessary for instituting apomixis which is accomplished through bulbifery. To begin with, the bulbil initials co-exist with the flowers, later their development accelerates and within a few weeks the entire head is packed with bulbils. As the spathe ruptures the bulbils produce leaves and the heads turn green. Each bulbil functions as a potential propagule capable of differentiation into a complete plant. In such clones, flowers represent remnants of the sexual apparatus that have become defunct over time.

GVALADZE (1968) has reported one more type of apomixis-agamospermy in some Russian cultivars. The aposporous embryo sacs have been reported to develop from the chalazal tissue of the nucellus and the integumentary cells. The initials of adventitious embryo sacs are distinguishable by their size, abundance of cytoplasm and large nucleus. These cells enter the prophase when the normal embryo sac is at the four-nucleate stage. The aposporic embryo sacs have been observed (GVALADZE 1968) but whether they survive to maturity is not known. GVALADZE (1968) states that apospory is a manifestation of the disruption of normal sexual reproduction. He states emphatically that even such forms of garlic where female gametogenesis proceeds normally are in reality apomicts as well.

The precise cause behind the substitution of sexuality by bulbifery in the face of normal meiosis (at least in some clones) is not clear. It seems that the inability of cultivated garlic to propagate by seed has been exploited by the agriculturists in order to ensure uniformity in the performance of the crop. With the elimination of sexual recombination, cultivated garlic behaves as an obligate apomict showing regularity in behaviour and uniformity in phenotype. The abortion of male and female gametophytes at different stages of development suggests that the various cultivars of garlic carry different genes controlling apomixis. Through the experimental manipulation of such genes, it may be possible to restore sexual reproduction, the loss of which garlic has been able to withstand through vegetative propagation and perennial habit.

Scope of genetic improvement in garlic

Despite repeated vegetative propagation during thousands of generations, garlic has accumulated a fair degree of variability either through gene mutations or structural alterations of chromosomes. Systematic evaluation of some Indian cultivars has brought to light morphoforms varying in such important characters as the number of cloves per bulb, size of individual clove, colour of scales, nature of leaf, presence or absence of exserted inflorescences, nature of inflorescences-whether totally vegetative or mixed, pungency etc. While manipulation of the quality constituents of bulbs through breeding will be of paramount importance, efforts also need to be made for increasing their quantity. Since garlic bulbs are used for culinary purposes and as raw material in drug industry, the selection criteria would vary according to these purposes. Thus, while strong odour would be a desirable quality for its use in the kitchen, it would be offensive in drugs.

Once quantitatively and qualitatively superior genotypes have been established, their maintenance and rapid multiplication through bulbifery, agamospermy and vegetative propagation is assured. There is obviously sufficient scope for genetic improvement in garlic.

Techniques for breeding improved garlic

The possibility of utilizing various techniques standardized for the improvement of various economically important plants need to be discussed for garlic against its mating system, and the problems peculiar to this crop. While interspecific hybridization has opened up new avenues for improvement in some crop plants and has successfully contributed to the development of radically new and better types, in garlic the complete exclusion of sexuality is a major handicap. It mitigates all chances of gene flow needed for the creation and isolation of newer and rarer recombinant types.

The pre-requisite for recombinant breeding as a means of improving garlic is induction of sexuality. KOUL & GOHIL (1970) attribute the break down of sexuality to the development of bulbils on the floral heads. The fast growing bulbils are believed to usurp the flowers of all the nutrition supplied by the vegetative parts. As a result, the flowers cease to grow and wither without producing any seed. If this is really so, fertility should be restored by the suppression of bulbifery at an early stage. This was attempted through the use of gamma rays and growth regulators and by picking the bulbils as soon as they became visible.

Commenting on apomixis in *A. carinatum* LEVAN (1937) advocated that apomicts arise from amphimicts by gene mutation. Since gamma rays are known for their mutagenic efficiency they were used to irradiate garlic bulbs with the hope of inducing a back mutation converting the apomict to its original amphimictic form. Of the 0.5, 1, 2, 3, 4 and 5 kR dosages used all treatments beyond 2kR proved lethal. 0.5 and 1kR treatments had no detrimental effect either on sprouting or on subsequent plant growth but the treated plants produced mixed heads like the control. Even development within the microsporangium did not proceed beyond the microspore tetrad stage indicating that irradiation could not be of much consequence.

Growth regulators like 2, 4-dichlorophenoxy-acetic acid, morphactin and benzyle aminopurine were sprayed on the inflorescence of *A. sativum* to assess their impact on bulbil growth. Although chemical treatment reduced bulbil size it did not help in advancing differentiation of gametophytes.

Comparatively better results have been obtained by manually picking bulbils from mixed heads. LEVAN (1937) used this technique with success for the induction of fertility in *A. carinatum*. In *A. sativum* repeated picking of bulbils helped in delaying the abscission of flowers; the flowers turned pink and even attracted pollinators. Despite these changes even this procedure could not help in inducing fertility. Nonetheless, these results definitely establish the fact that the development of flowers is affected by the development of bulbils.

The claims of KONONKOV (1953) with respect to the successful induction of fertility in garlic have not been confirmed by any one.

Recently, KÔNVICKA (1973) published an account stating that bulbifery in garlic is caused by infection of the plants with a mycoplasm. He claims to have succeeded in forcing the plants to set seed by treating mixed heads with antibiotics. NOVAK & HAVŘANEK (1975) repeated the experiment. They applied tetracyclin to the intact and detached inflorescences but obtained only shrivelled seed which failed to germinate. The report has yet to be confirmed. The occurrence of bulbifery in all cultivars, irrespective of the place where they are grown makes it unlikely that mycoplasm is involved in the imposition of apomixis.

Since restoration of fertility in garlic is no where in sight, there is little hope of accomplishing genetic improvement in this crop plant through hybridization. Somatic cell hybridization can provide a way out of this impasse. One can hopefully look forward to the utilization of this technique for combining good qualities of different cultivars into one.

If ever achieved, induction of sexuality can also pave way to the employment of inbreeding or selfing as a means of breeding improved garlic. Considering the heterozygous nature of the crop, selfing undoubtedly offers good scope for exposing the locked up variability for selection. However, induction of fertility alone will not do to ensure selfing because alliums in general are protandrous with male gametophytes getting released from the microsporangium, while the megaspore mother cell has just entered into reduction division. Manipulation by way of pollen storage etc., will therefore also have to be resorted to.

Polyploidy as a tool in breeding superior garlic

The practice of vegetative propagation and the desirability of improvement in the vegetative organs, particularly the bulb, constitute special advantages in persuing polyploid breeding in this crop plant. The advantages of polyploidy, if any, could readily be exploited since maintenance and propagation of polyploids pose no problem. Since no natural polyploids or aneuploids are known in garlic, the polyploid-breeding-programme has to start with induced polyploidy. The evaluation of yield and other qualities will have to be done through the assessment of a diverse range of genotypes with doubled chromosomes.

Mutation breeding

On account of absolute apomixis and the improbability of employing recombination breeding, specific defects in commercial varieties of garlic might be rectified using mutation breeding. Even for purposes of inducing resistance to common fungal and insect pests, mutation breeding needs to be fully tapped so that some otherwise good varieties can be rehabilitated for commercial cultivation. Apomixis has a factor that is extremely favourable for the improvement of this species in the hands of a mutation breeder. The mutations are fixed more rigidly by apomixis than amphimixis.

Clonal selection

Systematic evaluation of naturally available genetic diversity needs to be undertaken for improving garlic. Since the plant is an exotic introduction, though an old one, genetic diversity in Indian cultivars is inadequate to meet the demands of breeders. This has to be compensated by raising collections from the centre of origin which lies somewhere in or around Afghanistan. As mentioned before, clonal variation exists in respect of bulb characteristics such as the number of cloves per bulb, size of individual clove, odour etc. Since these characteristics are of direct importance, a well conceived selection programme is likely to yield good dividends. It follows from the above discussion that although a seemingly conservative crop plant with a closed type of breeding system, garlic offers some avenues for improvement provided these are opened up for breeding through research.

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