SELF INCOMPATIBILITY, MALE STERILITY
AND POLLINATION MECHANISM IN NIGER
{GUIZOTIA ABYSSINICA (L.F.) CASS.}
- A REVIEW

H.S. Patil and S.S. Duhoon

All India Coordinated Research Project on Niger,
Zonal Agricultural Research Station, Igatpuri - 422 403, India

ABSTRACT
Breeding schemes are ordinarily based on natural and/or controlled mating system in a crop species. The presence of sporophytic self-incompatibility presents hurdles in the yield improvement programme of niger. The practical application of self-incompatibility in niger breeding is still unexplored. Thus, the work on these aspects is reviewed and discussed in this paper. The constraints and strategies for improvement of yield in niger crop are suggested.

1 Present address: AICRP on Sesame and Niger, J. N. K. V. V. Campus, Jabalpur - 482 004.

Niger {Guizotia abyssinica (L.f.) Cass.} is an important minor oilseed crop of tropical and subtropical ecosystems. Among the important niger growing countries viz., India, Ethiopia, East Africa, West Indies and Zimbabwe of the world. In India, niger crop is grown on an area of about 4.48 lakh hectares with the annual production of 1.152 lakh tonnes (2000-2001). It is cultivated mainly in the states of Orissa, Maharashtra, Madhya Pradesh, Bihar, Karnataka and Andhra Pradesh and to some extent in hilly areas of Rajasthan, Uttar Pradesh, Gujrat, Tamilnadu, Assam, and also in some parts of North Eastern Hills states of the Country.

Niger yields high quality edible oil with pleasant nutty sweet taste. Niger is mainly used for oil extraction (about 70 per cent) for culinary and culinary purposes. Its oil is bluish white in colour and is a good absorbent of fragrance of flowers and thereby it is used as base oil in perfume industry. As reported by Vles and Gottenbos, (1989) and Dagne and Johnsson, (1997), it is well known for the dietary fats being rich in linoleic acid which prevent cardiovascular disorders, such as, coronary heart diseases, sclerosis and high blood pressure and also linoleic acid derivatives serve as structural components of plasma membrane and as precursor of some metabolic regulatory compounds. Niger oil is very much beneficial to human being (Fujimato et al., 1990). Besides, its use as an important edible oil in Ethiopia and India, it is also used for manufacturing of soaps, paints and illuminants in the industry of these countries (Riley and Belayneh, 1989). Niger is consumed about 18 % in certain regions as food in the form of chutney mixed with chilly and spieces. Niger seed is a good bird feed.

Niger, a naturally selected mutant of Guizotia scabra subspecies Schimpheri is highly cross-pollinated crop. Cross-pollinated crops are endowed with floral mechanism, which ensures cross-pollination through vectors mostly honey bees. Niger plant has developed homomorphic pretenders of self-incompatibility mechanism to get rid of any degree of self-pollination. In view of scanty and scattered literature available in niger, it was felt to review the research done on self-incompatibility, male sterility and pollination mechanism in niger. This paper presents the discussion of the past research on various mechanism favoring autogamy in niger and suggests the formulation of a new breeding programme for improvement of this crop.
Self-Incompatibility

In many species (over 300 species of about 70 families), active and functional pollen grains fail to effect fertilization on self pollination followed by no seed set, an exclusive event on cross pollination. This phenomenon is called self incompatibility, which was firstly described by Koelreuter. In self-incompatibility, pollen grains may fail to germinate or enter the stigma. Pollen tube may grow too slowly in the style to effect fertilization before the flower drop, or the embryos (if fertilization does take place) degenerate.

A highly evolved and effective mechanism controlling fertilization is self incompatibility, which was defined by Netton De court (1977) as the inability of a fertile hermaphrodite seed plant to produce zygote after self pollination. Pollen may be normal, viable and fully functional on another genotype but, self fertilization is prevented. Because of self-incompatibility systems which are based upon the inherited capacity of a flower to reject its own male gamete, the system includes post-fertilization failures due to zygotic inviability, embryo abortion and endosperm abnormalities (Hayman, 1956).

Crowe (1953) studied the incompatibility system of compositae family in Cosmos and found that the dominance relationship of incompatibility genes in this family did not confirm to any system.

Gerstel and Rinner (1954) reported self-incompatibility in few genera of compositae family, and found that in some members of this family, the incompatibility alleles showed a sporophytic determination of their behaviour in one parent and gametophytic in other.

Briggs and Knowls (1967) first reported whorled phyllotaxis in niger with the frequency of plant in the range of 0.00 to 0.36 percent. Mass selection in cultivar "Ootacmund" increased the frequency of whorled plants from 0.36 to 23 per cent at the end of first cycle of selection and 28 per cent at the end of second cycle of selection. There was no inertia to advance under selection as the whorled plants were identified early transplanted in isolation and allowed to intercross there.

Naik and Panda (1968) found niger (Guizotia abyssinica) is as a self incompatible crop. It is a member of compositae family and nothing has been reported so far on the genetics of self incompatibility in niger.

Panda and Rao (1970) studied the effect of hot water treatment and ultra violet irradiation on the self incompatibility mechanism in niger. Styles were immersed in hot water of varying temperatures and then self pollinated. Growth of pollen tube in styles was noted and compared with control. Maximum pollen tube growth was seen in styles, when treated with hot water of 45°C. Higher temperature proved lethal and lower one ineffective. Therefore, they have suggested that this temperature bring forth some biochemical changes in inactivating the incompatibility mechanism. An exposure of styles to ultra violet irradiation for 10 and 15 minutes, showed increased pollen tube growth as compared to its control. Thus, they have concluded that hot water or ultra violet treatment inactivated the incompatibility mechanism. This inactivation might be due to some biochemical changes in the inhibiting substances.

Basantia et al. (1975) studied the floral biology of niger with reference to self incompatibility. Pollination and fertilization studies with particular reference to pollen viability and stigma receptivity in five varieties of niger revealed the self incompatible nature of this crop. The occurrence of trinucleate pollen grains in the protrandrous disc florets suggested that the seed of self incompatibility
was possibly located in the stigma.

Panda and Rao (1976) found that the self incompatibility in niger was governed by multiple oppositional alleles at one locus. Here the pollen reaction was determined sporophytically. Some alleles showed dominance and some behaved as recessives in pollen or stigma. They reported five ‘S’ alleles, of which three (S1, S3 and S5) were in active form and two (S2 and S4) in recessive condition. Two types of allelic relationship, namely, independence and dominance were detected in heterozygous state. Of the five alleles recovered in progeny families, three were independent but dominant over the other two in pollen and stigma. The activity of alleles was similar in hetero and homozygotes.

Janoria and Verma (1978) observed a few plants having whorled, instead of the normal decussate, phyllotaxis in the cultivar ‘Ootacmund’ during kharif 1974 season. The whorled plants bear three leaves per node. The whorled character was somewhat restricted in expressivity. Nearly 5 per cent of all the whorled plants showed, three leaves on one or more, but not all nodes. They observed response to selection, which is indicating the presence of genetic modifiers restricting penetrance of the whorled character. On an average, whorled plants gave 10.11 per cent increased seed yield. This yield advantage might be attributed to 30 per cent increase in total photosynthetic area, which in turn, supported 15 per cent increase in the number of capitula per plant, though somewhat reduced size.

Gaur and Singh (1983) screened 144 genotypes of niger for degree of self incompatibility. On selfing, seed setting ranging from 1 to 6.6 seeds/capitulum was noted in 17 genotypes. The remaining genotypes were found to be highly self incompatible (seed setting less than one seed/capitulum). A comparative study of pollen tube growth in the style was carried out by means of fluorescence following compatible and incompatible pollination. They discussed different processes of the physiological and genetic break down of self incompatibility.

Sinha et al. (1993) studied the degree of self compatibility among twenty genotypes of different maturity group. The degree of self compatibility varied with the genotypes and maturity groups. It was least in RCR-290 and considerably high in RCR-64 and No 71 followed by Ottacmund, ON-2 and SMGN-1 genotypes. Therefore, they, suggested the need of screening of a large number of genotypes of niger for self incompatibility.

Sujatha (1993) studied the pollen recognition-rejection phenomenon and devised appropriate method to overcome the self incompatibility barrier. Stigma receptivity and stage of pistil controlling intraspecific incompatibility were tested in a series of self and cross pollination at various stages of pistil development with fresh pollen in conventional emasculation at all stages and the day of anthesis by refined crossing method of Sujatha and Angadi (1989). The pollen-stigma reactions were studied with pistils fixed in 3 per cent glutaraldehyde in 0.1 m phosphate buffer (7.2 ph) under scanning electron microscope. The efficacy of various treatments viz., bud pollination, delayed pollination, stump pollination and temperature effects in surmounting the incompatibility barriers were assessed.

Stigma receptivity in terms of per cent seed set confirmed the nonreceptivity of stigmas in the buds, while the highest seed set was recorded with the stigmas pollinated 24 hours after anthesis (77.77 per cent), followed by day of anthesis (70.90 per cent) and ray florets (43.67 per cent). No seed set was evident when stigmas still enclosed in the buds were pollinated. The pistils at all stages of development failed to promote seed set after
self pollination. This indicated the self incompatible nature of population of niger and absence of preanthesis pollination. Scanning electron microscopic observations proved the functional nature of protandry operating in niger. Of the various treatments tested in overcoming the incompatibility barrier, high temperatures and stump pollination methods were effective. The maximum seed set of 71 per cent under selfing at high temperatures of 50°C to 56°C was observed. The high temperature may be weakening the 'S' reaction products as most of the enzymes are heat labile, as in case of Brassica crop.

Nemissa et al. (1999) studied the self incompatibility system in Ethiopian population of niger. The experiment, involving 1425 pollination, were carried out both by petridish technique and in-situ pollination of heads. The extent of pollination was recorded. In most cases, this sporophytic self incompatibility was believed to be controlled by a single 'S' locus (the recording of four self incompatible mating groups) in the population of Guizotia abyssinica studied. An additional 'S' locus, 'I', was proposed for this crop, based on the presence of more than four self-incompatible mating groups in most of the plants studied. Pseudocompatibility and seeds obtained from Pseudocompatible crosses were characterized. The self incompatibility system in Guizotia abyssinica is characterized by the presence of two and one way incompatibility, reciprocal differences and self compatibility. Natural selection might favour the establishment of self-compatible genotypes in the populations of Guizotia abyssinica. The frequency of self compatible genotypes showed variation of varying magnitude in populations originating from different localities.

Patil (2003) studied the pollination behavior under different kinds of bagging (muslin cloth, craft paper and oil paper bagging) as well as under open pollination and reported that bagging had different role in seed setting in open pollination. The bagging of individual bud had an adverse effect on seed setting which ranges from 1.67 seeds to 2.41 seeds per capitulum. Different genotypes behaved differently for seed setting in different types of bagging. Oil paper bagging on an average was superior than other bagging for seed setting. Significant reduction in seed setting was observed in musline cloth bagging. The genotypes viz., JN-124, No-71, JN-66 and ON-2 were good seed setter under different types of bagging and thus, these genotypes was recommended for inclusion in the niger improvement program for yield enhancement as well as development of hybrids.

Pollination Mechanism

Manifestation of heterosis and its commercial utilization in castor and sunflower encouraged the Niger breeder too, to develop hybrids in this minor oilseed crop. Generally for emasculation and pollination following three methods are followed by the breeders.

1. Hand emasculation and pollination
2. Gametocidal emasculation and pollination
3. Male sterility and pollination

1. Hand Emasculation and Pollination: The floral structure of Niger is such that production of hybrid through this method is tedious, time consuming and costly. In this method, all disc florets being bisexual are removed with the help of forceps just prior to the opening of the capitulum leaving ray florets only. Ray florets are pistillate as this number are 6-8. Emasculation (removal of disc florets) is done during evening 3 to 5 PM. Emasculated capitulum is bagged with butter paper bag. Next morning, pollens of desired parent are collected from bagged parents and dusted on ray florets followed and repeated twice to ensure the fertilization. In this method, seed setting is hampered owing to injury caused during removal of disc florets from capitulum.
Niger Research Center at Chhindwara is actively engaged in utilization of this method.

2. Gametocidal Emasculation and Pollination: Gibberallic acid is well known gametocide, when applied during gamete differentiation in the floral bud. It restricts the differentiation of antheridium. Experience of sunflower crop is utilized in this method. 100 ppm GA spray at Pinhead stage is quite effective to induce male sterility in the florets. Later on, the pollens of desired parents are dusted. This process of pollen dusting is done in the mornings for four consecutive days, as being centripetal flowering in the capitulum and takes 3-4 days to complete. Niger Research Project at Igatpuri is actively engaged in utilization of this method.

3. Male Sterility and Pollination: Genetic male sterility was first observed and identified at Birsa Agriculture University, Kanke, Bihar now in Jharkhand State, India, by Dr. Trivedi during 1984. The exploitation of heterosis in Niger, a Herculean task in the past, has now been feasible due to presence of male sterility (Trivedi and Haider, 1985). The male sterility observed is genetic in nature and governed by recessive alleles, which are closely linked with legulate homomorphic florets in the capitulum. This will be discussed in detail under male sterility heading.

Naik and Panda (1968) studied bud pollination in one day old to seven day old buds in six varieties of Niger. As control, 10 capitula in each variety were allowed to self inside polythene bags. Natural selfing did not produce seed setting at all. In contrast, bud pollination gave a progressive increase in seed setting from one day old to three day old buds, in four out of six varieties studied. Pollination made to buds older than three days are found to be completely sterile. This increase in self fertility in Niger might be either due to absence of inhibitory substances or insufficient concentration of inhibitory substance in the young style.

Shrivastava and Shomwanshi (1974) studied selfing, crossing technique and extent of cross pollination in Niger. High amount of cross-pollination, almost 100 per cent was observed. Selfing with parchment paper bags reduced the seed setting drastically i.e. 0.2 seed/capitulum as compared to 40-60 seeds/capitulum in open pollinated condition. Rubbing the capitulum daily for 3-4 days after opening with soft paper edge in order to spread the pollen grains on the stigma of the same head improved the seed setting to 2.4 seeds/capitulum under selfing. Dusting the capitulum with pollen grains of male parent without emasculation for 3-4 days after its opening was found to be safe and easy technique for crossing.

Jambhale and Misal (1977) studied seed setting in various selfing methods. Muscline cloth bags and butter paper bags were used for selfing. Methods of selfing adopted were 1. Selfing individual heads, 2. Selfing individual plants and 3. Selfing two plants in a bag. Selfing individual plant or selfing two plants in a bag of muscline cloth produced approximately two seeds/head. Selfing individual head or individual plant or two plants together by butter paper bags had no seed setting or very poor seed setting. Selfing individual plant or two plants together by muscline cloth bag seems to be good method. However, seed setting is considerably low as compared to open pollinated crop.

Ramachandran and Menon (1979) studied the effect of different methods of pollination as furnished below.

1. Encasing the capitula with butter paper bags.
2. Mantling whole branches in muscline bags.
3. Rubbing the surface of capitula gently with the thumb.
4. Actual dusting of the pollen from flowers
of a plant of the stigma of the other capitula borne on the same plant.

5. Sib pollination was effected by deposition of pollen collected in bulk on the capitula of sister plants of the same line and also enclosing branches of unopened capitula of sister plants of variety inside fine muslin cloth bag.

They observed 55.2 per cent seed set by open pollination and 24.1 per cent seed set by artificial sib pollination by utilizing butter paper bags.

Sujatha and Angadi (1989) have studied the floral structure and crossing methods. The stamens in niger are epipetalous and syngeneicous. At the time of opening (around 8 am on clear sunny day) the disc florets appear swollen and turn yellow. The anther column becomes more conspicuous with white tip. This is the proper stage for emasculation. This white tipped anther column is to be pulled out by way of quick jerk with fine tweezers. The syngeneous anther tube comes off quickly and without any damage to stigmatic surface as the latter is at lower level than the anther tube. Pollinate these florets with desired pollen grains. This improved method has registered 81 per cent seed set. The reasons for enhanced seed set in this revised method over the usual crossing method could be due to i) full exposure of stigmatic surfaces early in the day and thus allowing more time for pollination and ii) minimal mutilation or injury.

Sinha et al. (1994) studied seed setting under different conditions of bagging viz., fine muslin cloth, craft paper, butter paper and open pollination in 20 varieties of niger. Bagging as a whole, had an adverse effect on the seed set which ranged from 1.29 to 2.39 seeds. Observations revealed that seed set was more under craft paper and least in muslin cloth in early varieties while in late varieties it was more under butter paper and least in muslin cloth. High humidity restricted air circulation and high temperature during monsoon hindered good seed setting in case of early maturing varieties. But, this was not true for late varieties, which flowered at the fag-end of the rainy season.

Male Sterility

Niger is 100 per cent cross-pollinated entomophilous plant with sporophytic protandrous self-incompatibility. The artificial crossing in niger is very tedious owing to tiny bisexual florets congregated in the capitulum. Male sterility has been utilized on large scale for development of hybrid in many crops. Therefore, it is essential to identify and exploit male sterility system. Only a few reports are available on male sterility in niger. Those are being discussed here.

Hooker (1978) described the inflorescence of Niger having heteromorphic capitulum with two types of flowers. Disc florets are centripetally arranged, small tubular pentameric and bisexual, while ray florets are unisexual, legulate pistillate. The niger crop is a highly cross pollinated entomophilous crop with protandrous self-incompatibility.

Trivedi and Haider (1985) observed male sterile plant in gynoecious condition in the population of a variety N-5 during 1983. This plant was phenotypically similar to normal plant and produced five homomorphic capitula.
All florets of the capitulum were pistillate and ligulate in nature with bifid or tetrafid stigma. Forced selfing failed to produce any seed, however, 25 half-sibbed seeds were obtained on open pollination. Those seeds were sown to know the prepotency of gynomonoecism. Out of 25 seeds sown, 20 germinated, 10 of which were homomorphic legulate gynomonoecious, while the rest produced normal heteromorphic capitula, suggesting male sterility might be controlled by a nuclear gene, which is considered to be the first report in niger.

Trivedi and Sinha (1986) observed one male sterile plant in gynomonoecious condition in the population of niger. This plant was phenotypically similar to normal fertile plant, but had large sized buds. Critical examination of capitulum revealed that whole capitulum was homomorphic with only pistillate florets. The centrally situated bisexual, tubular disc florets found in normal condition, were all modified in to legulate pistillate florets with bifid or tetrafid stigmas. Androecium was either absent or rudimentary in development. Although in some of these central florets empty reinform anthers, devoid of pollen grains were observed; selfing failed to produce any seed. Nevertheless an open pollinated 25 half sibbed seeds were obtained. These half sibbed seeds were sown in next generation. Only 18 seeds out of 25 seeds germinated. They were vigorous in growth. Nine of them showed homomorphic legulate gynomonoecious condition and the rest produced heteromorphic capitula with a outer whorl of 8-10 pistillate ray florets and inner centripetal whorls of tubular bisexual disc florets. The segregation pattern in two types of capitula, thus indicated to be of genetic in nature. Its exact genetic nature needs further investigation.

Singh and Trivedi (1993) has made attempts to quantify the magnitude of heterozygosity response among the crosses obtained through genetic male sterile lines and to identify the best hybrid over better parent and the commercial variety of the region N-5. Heterosis was significantly high for seed yield and its attributes in two crosses viz., GMS x RCR 290 and GMS x Phule 1. Thus hybrids may be an answer to have a quantum jump in niger.

Sujatha (1997) reported direct adventitious shoot regeneration from in vitro cultured leaves of male sterile plants for effective maintenance of 100 per cent male sterility in niger. The system also eliminates the need for identification and maintenance of maintainer, which is difficult to detect in heterozygous state due to predominantly monogenic recessive nature of ms gene. This relatively simple micro propagation method also has potential application for Niger breeding, elite clonal propagation, cell selection for desirable mutants and genetic transformation.

Niger crop by virtue of its enormous variability to various characters of economic importance offers tremendous scope for improvement through heterosis breeding (Anonymous, 1999). F1 hybrid seed production necessitates the use of hybridization control system. Though self-incompatibility coupled with protrandry has been reported as the out-breeding mechanism, it was found to be unstable at higher temperatures. A stable male sterile mutant with altered but attractive floral phenotype was induced through gamma rays (20 to 100 kr). Rouging of male fertile plant is Labour intensive and reduces plant population in seed field. It is also difficult to maintain male sterile plants due to lack of maintainers. A rapid and efficient method of in vitro plant regeneration for large-scale propagation of male sterile plants of niger had been developed. The method facilitates effective maintenance of 100 per cent male sterile plants against 50 per cent in normal breeding procedures.
The system also eliminates the need for identification and maintenance of maintainer, which is difficult to detect in heterozygous state due to predominantly monogenic recessive nature of ‘ms’ gene. The method developed constitutes the first report of whole plant regeneration from mature tissues in Niger.

On critical examination of all above discussed reports of different research workers, it is felt that the nature and control of available existing male sterility is not clearly understood as yet and thus, it needs further detailed systematic study on this aspect in Niger. Fortunately, if the scientists succeed in this invention, it will be a golden day for niger improvement program. We expect success in near future in this regard and will prove that this is not a dream.

Constraints limiting production and productivity: In spite of its importance as an edible oilseed, the cultivation of this crop is confined to marginal lands of tribal areas under very poor agronomic practices resulting in low average yields. Hence, low yield of this crop is not surprising. Limited cultivation of this crop to productive regions may not be feasible at present. Efforts should be made to identify the causes of low yield in different areas and conditions of cultivation of this crop and seek ameliorative manners.

One of the most important causes for low yield is the sparse plant population of the crop, which may be due to several reasons including improper seed bed, broadcasting the seed in the field etc. Very frequently natural calamities are so bad that the farmer is hardly able to harvest 50 to 60 per cent of the sown area. Thus, even with the improved varieties, the production is very low.

Niger being grown mostly as Kharif crop, suffers in early stages due to excessive rains and non receipt of rain at later stage of grain filling and developmental stage, which result in low production. Rain during pollination period also hinders pollination resulting in empty capsule without seed set.

Constraints and Future Thrusts:
Niger, being considered as an oilseed crop of tribal or poor farmers, has also attracted considerable importance in the context of present day shortage of edible oil. Improvement in this highly cross pollinated crop may be accelerated if the following constraints and strategies are considered in the improvement program in Niger.

1) The presence of self incompatibility in niger hinders isolation and maintenance of inbred lines, a prerequisite for the exploitation of hybrid vigour. To identify a type with minimum extent of self-incompatibility through critical screening of germplasm collected extensively either from India or abroad as suggested by Nemomissa et al. (1999).

2) To resort for mutation to break self incompatibility in niger to enhance the stigma receptivity and its synchronization with pollen dehiscence.

3) With the success on above two aspects, it would be possible to develop composite/synthetic suitable for different niger growing areas of the country, by adopting suitable recurrent selection procedure.

4) Development of high yielding inbred line/varieties is only possible through intensive hybridization between distantly related types. Improved varieties so far evolved are selection from local materials with a narrow genetic base or through intra/inter population improvement program by exploiting self incompatibility in niger. These procedures are helpful in breaking the barriers at each plateau level.

5) With the advent of genetic male sterile line developed and isolation of potential inbred lines, exploitation of heterosis in niger would be possible; though considered as "Herculean
The male sterility obtained is genetic in nature and governed by recessive gene, which is closely linked with legulate homomorphic florets in the capitulum; while capitulum consisting of heteromorphic ray florets and disc florets are fertile. This has opened a new dimension of heterosis breeding in niger improvement.

6) Convergent improvement program be followed for combining gradually all yield contributing characters. Though this approach is time consuming and requires sufficient patience and care right from the onset, yet it provides real breakthrough in expected manner in the long run. Desirable lines obtained at different stages of improvement of base population could out yield and released for general cultivation.

7) More active involvement of non governmental organizations established for benefit of tribal peoples in research and development is needed.

8) The most important point to be considered is training to research personnel in advance breeding techniques, agronomic practices and their management. There should not be a financial constraints in this regards, as the only very few scientists are working at present in this crop. This will accelerate the niger research program very fast with sweet fruits.

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