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Breeding True Cassava Seed Progeny for Mosaic Disease (CMD) Resistance

By

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Keywords: **Cassava; Cassava Mosaic Disease (CMD); propagation; seed multiplication; Sexual seed**

Abstract

The propagation of cassava through true seeds (sexual seeds) rather than by clones is a promising option due to its manifold advantages like enhancing the multiplication rate, longer seed viability, ease of storage and transport. The high genetic heterogeneity and consequent variation among seedlings is the major stumbling block in sexual propagation. In the present study, a promising cultivar .Ambakadan. with profuse fruit setting, seed output and male sterility was identified. Nursery technique and agrotechnique were standardized for the establishment of a seed crop. Open pollinated and hybrid progenies of .Ambakadan. were evaluated for important economic traits. Tuber yield at first clonal stage (C1) was significantly superior to that of the seedlings. By providing higher plant population at seedling and first clonal stage, yield levels could be enhanced. The dry matter content , starch output,HCN of seedlings and first clones were comparable to that of the commercial varieties. In order to breed more homogeneous, high yielding, high starch sexual progenies having high level of mosaic resistance, hybridization of .Ambakadan. with fourteen male parents were undertaken. A CMD resistant line was used as one of the male parents. Evaluation of the hybrid progenies at seedling and first clonal stage along with clones of high yielding varieties as control indicated the superiority of the cross involving the CMD resistant line over the others in respect of CMD infection. A breeding strategy has been evolved to generate more homogeneous CMD resistant sexual progenies .

Introduction

Cassava has enormous potential in developping countries particularly India for poverty alleviation and food security due to its ability to grow well in marginal and waste lands under poor management and its capacity to yield well even under such unfavourable conditions. The slow multiplication rate under clonal multiplication, bulk of cuttings material and the accumulated bacterial germs year after year are the major impediments which prevent the rapid spread of the crop in far-flung poverty stricken areas of the country.

In case of the traditionally propagation by vegetative means, the propagation rate is only about ten to fifteen times. Because of this slow rate and difficulty in transporting the planting materials to distant places, the crop could not make much impact either as a food crop or industrial one in the country.

Initial studies on cassava production from sexual seeds were done by Nassar and Ohair (1985) They suggested that the use of true seed in place of stem cuttings for cassava production would eliminate these problems and potentially reduce production costs. One limiting factor though , is lack of quick and uniform seed germination. To reach this objective they developed cassava clones with early germinating seed so that it be used in plant breeding techniques such as mass selection. This may gradually modify cassava population characteristics. Propagation of cassava by seed would serve as filter for the accumulated bacterial germss present in given plantation. Few fungal and bacterial agents can be disseminated in sexual seed, but simple and effective treatments have already been developed for their eradication (Lozano & Nolt, 1989). The open pollinated progenies of even highly infected parents would be free of CMD at the initial stages but subsequently succumb to the disease due to secondary infection.

The major hurdles in the commercial adoption of true seeds are due to high level of heterozygosity among the seedlings and the inherent susceptibility of the progeny to CMD infection. In this context the germplasm accession MNga-1 developed by IITA assumes importance. This accession was initially developed by IITA Nigeria and had shown stable resistance to CMD over three planting season in Nigeria. (Fokunang, C.N.,2000). It showed stable resistance to CMD in many field trials conducted at Central Tuber Crops Research Institute (CTCRI) and All India Coordinated Research Project (AICRP) centres during 1995-2002.

In the present study, an attempt has been made to standardize the sexual seed propagation and to identify the most preferable parents which are capable of generating more homogeneous progenies having high level of CMD resistance.

Materials and methods

Detailed studies on TCS were undertaken at Central Tuber Crops Research Institute during the period 1988 to 2002. Field experiments *viz.,* seedling evaluation and subsequent clonal evaluation were carried out at Central Tuber Crops Research Institute, Thiruvananthapuram (latitude 8^0 32. N, longitude 76^0 55. W), TNAU Coimbatore(latitude 11^0 N, longitude 77^0 E), IGAU Jagadalpur (latitude 19^0 05. N, longitude 82^0 02. E) and at Agricultural Research Station, Peddapuram (latitude 18^0 59. N, longitude 78^0 55. E). In field experiments the recommended manurial schedule of 12.5 t ha^{-1} FYM as basal and NPK doze of 100:50:100 kg ha^{-1} was adopted. Starch content of the tuber was estimated using the specific gravity method,(Bainbridge,Z *et al*.,1996). The methodology adopted for the estimation of dry matter content also listed in Bainbridge,Z *et al*.,1996. The HCN content of the tuber were determined by spectrophotometric method. (Nambisan, B & Sundaresan,S 1984).

Experiment I- Seed output

Nine hundred and ninety five germplasm accessions were screened to identify profusely seed bearing accessions under open pollination. Mean number of fruits per plant and weight of 100 seeds were noted.

Experiment II- Seed storage and germination

Studies on seed storage were conducted by storing well dried seeds under ambient conditions and examining the germination percentage at an interval of two months upto 12 months.

Studies were also conducted to assess the effect of different chemicals on enhancing germination and sprouting. Open pollinated sexual seeds of .Amabakadan., dried and stored under ambient conditions for four months, were treated with different concentrations of chemicals. The treatments were Water(control),Thiourea-0.5 per cent, Thiourea-1.0 per cent, KNO3-0.5 per cent, KNO3-1.0 per cent, GA3-100 ppm,GA3-300 ppm,GA3-500 ppm. The seeds were soaked for one day and two days with the same treatment. Seed germination and seedling vigour were recorded.

Experiment III- Establishment of seedlings in field

In situ sowing . in a well prepared field two seeds each were sown per site, 5cm deep at a spacing of 90 x 90 cm. Thinning was done after one month retaining one plant per site if both have established. The field was irrigated twice weekly for the first two weeks if sufficient rain was not available at the time of sowing and there after grown under rainfed.

Tranplanting from nursery - the primary nursery was formed in well prepared soil, and seeds were sown sufficiently deep preferably in raised seed beds at closer spacing in rows of 15 cm apart. The nursery was irrigated on alternate days for the first two weeks and thereafter at weekly intervals. The seeds started sprouting by 12-14 days and completed in about 3 weeks. A light dose of fertilizer @ urea -20 g; super phosphate - 30g; muriate of potash - 25 g for 10 square meter was given to the plants after 1 month of sowing. The seedlings were ready for transplantation after 45 days of sowing. The effect of removing tap root while transplanting in the field was also studied.

Experiment IV -Incidence of Cassava Mosaic Disease

The disease incidence among 10 OP seedling progenies and first clonal progenies was studied during 1992-93 and 1993-94 seasons at CTCRI, Thiruvananthapuram. Percentage infection was noticed in seedling progenies along with clonal population of M-4 and Sree Visakham. CMD incidence was assessed based on visual symptoms on each leaf of each plant. Entirely symptomless plants were recorded as CMD free and the rest as infected.

Experiment V- Evaluation of seedling progenies under two spacings

Seedling progenies of the ten parent.s *viz*., Ce 595, Ce 639, Ce 647, Ce 2, Ce 630, Ce 453, S_1, S_2, S_3 and Ambakadan were raised in primary nursery and transplanted to the main field after clipping tap roots. The experiment in the main field was laid out in a randomized block design with three replication in a plot size of 4.5 m x 5.4 m during the year 1994-95. The trial was harvested at 10 month stage. Observations were recorded on number of tubers, mean tuber weight, tuber yield and harvest index. The trial was laid out under two spacings *viz*., 70 x 70 cm and 90 x 90 cm.

In another trial seedling performance of 'Ambakadan' and TCH-3 was assessed with parents during 1998-99 based on tuber yield, tuber dry matter content, tuber HCN content, and tuber fiber content and cooking quality at 6^{th}, 7^{th} and 8^{th} month stage.

Experiment VI-Spacing cum Fertilizer trial

The trial was conducted at CTCRI, Thiruvananthapuram during 1996-97 with first clonal plants of .Ambakadan. OP seeds in a factorial experiment in randomized block design with three replication with a plot size of 4.5 m x 5.6 m. There were 3 levels of fertilizers *viz.,* F_1 - NPK @ 100: 50: 100 kg ha^{-1}, F_2 - 75: 37.5: 75 kg per ha and F_3 - 50: 25: 50 kg per ha. and three spacings *viz.,* S_1 - 90 x 90 cm, S_2 - 75 x 75 cm and S_3 - 60 x 60 cm. The clones of .Ambakadan. with recommended package of practices were treated as control. Mean tuber yield (t ha^{-1}) and tuber dry matter content (%) among different treatment were recorded.

Experiment VII . Seedling yield Vs first clonal yield

To assess the tuber yield potential at seedling and first clonal stage, a field trial was initiated at Coimbatore, Jagadalpur and Peddapuram with seedling progenies of five varieties of cassava *viz*., .Ambakadan., CI-609, CI-639, Inbred-1, Inbred-2 during 1996-97 and respective first clones during 1997-98. The seedling yield and the first clonal yield were recorded.

Experiment VIII- Evaluation of hybrid progenies

Hybridizations were undertaken during 2000-01 with .Ambakadan. female parent and the following 14 promising varieties as male parents: Addukumuttan, Ethakkarupan, MNga-1, M-4, TCH-2, Sree Rekha, Sree Prakash, CI-732, Sree Vijaya, Sree Jaya, Sree Sahya, H-226, H-165 and H-97. The hybrid seedlings were planted during June 2001 at CTCRI, Thiruvananthapuram and ARS, Peddapuram along with the clonal population of .Ambakadan. and .Sree Prakash. respectively as control. The recommended package of practices was adopted. Observations were taken on the germination percentage plant height, number of tubers per plant and tuber yield. The incidence of the cassava mosaic disease was also noted at regular intervals. The seedling progenies were harvested during March 2002. The first clonal populations of the hybrids along with the controls were planted in progeny row trial in June 2002 at both the locations. Observations were taken on plant height, number of tubers per plant and tuber yield. The incidence of the cassava mosaic disease was also noted at regular intervals

All the data on different parameters were subjected to analysis of variance using Genstat (Genstat 5 Committee, 1997).

Results and discussion

Seed output

The mean number of fruits per plant varied from 16 to 168. The male sterile line .Ambakadan. which is a popular cultivar grown in Kottayam and Iddukki districts of Kerala had recorded the highest mean number of fruits per plant. The weight of 100 seeds showed a range of 9.0 g to 12.9 g among the selected lines. The following accessions were identified due to high seed output potential

male fertile: Ce 595, Ce 607, Ce 630, Ce 639, Ce 647
male sterile: Ce 2, Ce 209, Ci 453 , Ambakadan
inbreds: S1, S2, S3

Seed storage and germination

Cassava sexual seeds can be stored under ambient conditions upto six months without any appreciable loss of viability. There is a gradual loss of viability during 6 to 8 month stage and after 8 months, there is a sharp decline in germination percentage. Male sterile lines registered very good germination (80-90 per cent) Figure1. Similar studies conducted by Kawano et al., 1980 revealed that cassava seeds can be stored about an year under ambient conditions without any serious decline in viability, and much longer at low temperature and low humidity

Figure 1

Cassava sexual seeds do not germinate satisfactorily in laboratory under ambient conditions. No success could be obtained when cassava seeds were tested in petri dish lined with moist filter paper. Studies conducted in aluminium trays filled with pot mixture under ambient condition also recorded poor germination of less than 9 per cent only. But when the trays were fully covered by dark cloths and kept for two weeks, germination percentage of more than 50 per cent was recorded indicating that the seeds need dark condition for proper germination. In the field good germination could be obtained after dibbling the seeds sufficiently deep in the soil or spreading a uniformly thick layer of soil after sowing the seeds.

Under the chemical treatments, s

Figure 2

eed germination and seedling vigour were high in seeds soaked for one day in 1 per cent KNO_3 and 300 ppm GA, Figure.2. Germination percentage of more than 60 per cent could be obtained at 17 days after sowing by treating the seeds for one day with the above chemicals. Transplanting period could be reduced to 30 DAS from 45 DAS by the above treatments. .

Establishment and survival of seedlings

Establishment and survival of seedlings of different parents ranged between 33.5 and 53.7 per cent when seeds were sown in situ compared to 90.0 per cent establishment among clones. Competition from weeds and unfavourable soil conditions during early stage of seed germination resulted in poor establishment and survival of seedlings. But when seedlings were raised in primary nursery and transplanted to the field, the establishment percentage was 75.4-91.5. (Table 1). The accession Ce 2 recorded the highest percentage of (53.7) establishment when sown in situ, and the accession ce 639 recorded the highest percentage of (91.5) establishment under transplantation from the nursery at 4^{th} month stage.

Removal of tap root

Table 1

Normally, the seedlings on growth and development give rise to well developed fibrous tap roots with very few undeveloped tubers. The tap roots are not edible. When tap roots of seedlings were totally removed while transplanting to the main field, the tuber development of seedlings were found to be quite normal. By adopting this simple technique clonal yield can be simulated at seedling stage itself.

Incidence of Cassava Mosaic Disease

Incidence of cassava mosaic disease among ten OP seedling progenies and first clonal progenies are given in Figure 3. The seedling progenies were less infected by CMD compared to the clones and recorded very low disease incidence of 1-4.2 per cent during the first couple of months. But subsequently the seedlings picked up infection rapidly and registered 10.0-28.8 per cent infection at fifth month.

Figure 3

Similar trend of higher CMD infection due to passage time was recorded in the first clonal progenies also wherein the level of CMD infection reached the level of control plots at 5 months stage. This indicates that just by adopting TCS technique it will not be possible to clean up the crop from CMD due to inherent susceptibility and secondary infection. Involvement of CMD resistant parent in the development of true seeds becomes all the more important in this context.

Evaluation of seedling progenies under two spacing

The results on number of tubers per plant, mean tuber weight, tuber yield and harvest index of ten OP seedling progenies along with control are presented in Table 2. The mean weight of tubers varied significantly among the entries. The clonal population of Sree Visakham at a spacing of 90x90 cm recorded the highest mean tuber weight of 0.284 kg $plant^{-1}$. Among the seedling progenies, .Ambakadan. and .CE-647. were

Table 2

identified as superior ones with 0.22 kg plant $^{-1}$ and 0.228 kg plant $^{-1}$ respectively. Seedling progenies of Ambakadan recorded a mean tuber yield of 15t ha^{-1} which was statistically on par with that of clonal population of Sree Visakham which recorded 18.70 t ha^{-1}. Closer spacing of 70 x 70 cm recorded significantly higher tuber yield as compared to that of the recommended spacing of 90 x 90 cm. Progenies of 'CE-647', 'S1' and 'Ambakadan' produced good yields at closer spacing and the yields were at a par with that of clones of promising varieties. The results indicate that by providing closer spacing to seedling and first clonal progenies the level of tuber yield per unit area can be increased considerably.

Detailed studies on tuber dry matter content and HCN content were conducted in the OP seedling progenies of .Ambakadan. and Top Cross Hybrid (TCH-3) and presented in Table 3. The figures in the parenthesis show the mean values for the seedlings and parents respectively. Dry matter content in Ambakadan seedlings increased from 6th to 8th month but in TCH-3, dry matter level maintained the same level in 6th and 8th month. In the seedling population of Ambakadan mean HCN content of tuber at 6th and 8th month were 55 g/g and 81 g/g respectively, whereas in TCH-3, the corresponding values were 30 g/g and 64 g/g. The HCN content at 8th month stage was generally higher possibly due to the drought spell. This indicates that the tuber dry matter and HCN content of the seedlings are comparable to that of the parents.

Table 3

Spacing cum Fertilizer trial

The results are presented in Table 4. The results indicated that closer spacing(60 x 60 cm) with NPK @ 100:50:100 kg ha^{-1} resulted in highest tuber yield (24.97 t ha^{-1}). The different levels of fertilizer did not show any significant effect on tuber yield and dry matter content. Tuber dry matter content in first clonal population was on par with that of control.

Table 4

Seedling yield Vs first clonal yield

The results of the trial conducted to assess tuber yield at seedling stage to that of the first clonal stage are presented in Figure 4. In all the three centres the first clonal tuber yields were significantly higher than the corresponding seedling yield for all the tested parents. This superiority of C_1 population over the seed crop indicates the scope of adopting first clones of promising parents for rapid spread of the crop.

Figure 4

Evaluation of hybrid progenies

The two major drawbacks of open pollinated sexual progenies have been high level of plant to plant variation and increased susceptibility to CMD in subsequent generation due to secondary infection. In order to overcome the major drawbacks hybridization programme was initiated with a promising female parent (.Ambakadan.) and 14 selected established varieties as male parents. The .Ambakadan. was identified as female parent due to the following reason

(i) High seed output

(ii) Being male sterile large scale production of hybrids possible by open pollination of selected male/ males in isolation

(iii) High tuber yield, dry matter, starch output and good culinary quality. It is a ruling variety in many parts of Kerala.

Among the male parents the exotic accession MNga-1 was included due to its stable resistance to CMD since its introduction at CTCRI during 1994.

The tuber yield performance of hybrid seedlings and the first clonal yield of the hybrids at CTCRI, Thiruvananthapuram and Agricultural Research Station, Peddapuram are presented in Table 5. At ARS Peddapuram the yield of first clonal hybrids were significantly superior to the corresponding seedling yield. At Thiruvananthapuram by and large, all the first clonal hybrid progenies recorded very low tuber yield which may be due to the prolonged drought during the year 2002-03. At Thiruvananthapuram, the hybrid progenies involving H-97,Adukkumuttan and MNga-1 as male parents recorded a first clonal yield of 10.56 t ha^{-1} , 11.16 t ha^{-1} and 10.86 t ha^{-1} respectively were statistically on par with that of the clonal population of .Ambakadan. as control (13.30 t ha^{-1}).

Table 5

The incidence of cassava mosaic disease was recorded at regular intervals and the percentage infection during seedling stage (6 months stage) and the first clonal (9 months stage) at Thiruvananthapuram and Peddapram are depicted in Figure 5 and 6 respectively. Among the male parents MNga-1 did not even record even a speck of CMD infection whereas all others were heavily infected by the virus. The above accession was tested at CTCRI farm since its introduction in 1994. This was also included the preliminary yield trial (PYT) of AICRP since 2001 (AICRP, 2002).All these years the plants did not take up CMD infection worth mentioning in any of the five AICRP centres except development of occasional transient symptoms at CTCRI which subsequently got eliminated naturally. The resistant character was further confirmed by grafting test with symptom expressing susceptible cassava root stock (CTCRI, 2003).

Figure 6

The first clonal hybrid progenies of Ambakadan x MNga-1 recorded the least CMD infection (15.2 per cent) at CTCRI Thiruvananthapuram. At Peddapuram the hybrids of MNga-1 did not show any symptom at seedling stage. At first clonal stage incidence of CMD was observed least and it was below 15 per cent. This along with the fact that the hybrid progenies involving .Ambakadan. and MNga-1 recorded high percentage of CMD free plants both at seedling and first clonal stage reflects the presence of a major gene/

genes conferring resistance to CMD in the male parent.

Recently the classical genetic analysis and molecular genetic mapping of highly CMD resistant lines of Nigeria revealed that a major dominant gene confers the resistance. Bulk segregant analysis (BSA) was used to quickly identify a simple sequence repeat (SSR) marker linked to the CMD resistance gene. A dominant gene for resistane to CMD has been found by conventional genetic analysis and molecular genetic mapping in a F_1 cross between resistant and susceptible parents. This is the first report of qualitative resistance to virus in cassava (Akano et al., 2002). The single dominant gene nature of the new source of resistance makes it useful in breeding for CMD resistance. The major gene nature also means that the genetic marker for marker assisted selection (MAS) can easily be identified.

The above mentioned host-plant resistance to CMD which was found in the third backcross derivatives of an interspecific crop between cassava and Manihot glaziovii to which MNga-1 also belongs gives strong indication of similar gene action in MNga-1 for CMD resistance. This unfolds greater scope for involving MNga-1 in large scale hybridization with the promising female parent .Ambakadan.. The cassava lines Ambakadan (female parent) and MNga-1 (male parent) have been identified to be the potential parents in the production of sexual seeds. A pollination block in isolation involving .Ambakadan. closely interspersed with MNga-1 left for open pollination should be able to generate large amount of hybrid seeds of the two parents(seeds collected from .Ambakadan. parent only). Stringent selection for rouging out all CMD infected plants in seedling and first clonal stage could provide a more homogeneous progenies of two promising parents having CMD resistant genotypes.

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Conclusion

The major hurdles in the wide spread adoption of the sexual seeds are due to the high heterogeneity of progeny and CMD susceptibility in subsequent clonal progenies due to secondary infection in field. The present study helped in the identification of two parents *viz.*, male sterile .Ambakadan.- the female and CMD resistant exotic accession MNga-1- the male. A pollination block in isolation involving .Ambakadan. closely interspersed with MNga-1 left for open pollination should be able to generate large amount of hybrid seeds of the two parents (seeds collected from .Ambakadan. parent only). The hybrid seedlings and first clonal progenies subjected to systematic rouging for CMD infection and undesirable plants can provide more homogeneous and CMD resistant population. The tuber yield, tuber dry matter, tuber starch and tuber HCN being at par with those of the commercially grown clones, the hybrid progenies from first clones onwards can be used for large scale cultivation in industrial areas and poverty stricken tribal areas of the country for food security.

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