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# Investigation on Foliar Spray of Nutrients, Growth Regulators on Hybrid Seed Quality and Genetic Purity Testing in Hybrid Pigeonpea [*Cajanus cajan* (L.) Millsp]

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**A B S T R A C T**

The experiment on effect of foliar spray of nutrients, growth regulators on seed yield and genetic purity testing in hybrid pegionpea [*Cajanus cajan* (L.) Millsp] was conducted at Seed Farm, College of Agriculture, Vijayapura, during *Kharif* 2019-2020. The treatment *viz*., foliar spray of control ($T_1$), 1% Pulse magic ($T_2$), 1% 19:19:19 ($T_3$), 0.2% Borax ($T_4$), 50 ppm NAA ($T_5$), 1% Pulse magic+1%19:19:19 ($T_6$), 1% Pulse magic+0.2% Borax ($T_7$), 1% Pulse magic+50 ppm NAA ($T_8$), 1% Pulse magic+1% 19:19:19+0.2% Borax ($T_9$), 1% Pulse magic+ 1% 19:19:19+50 ppm NAA ($T_{10}$), 1% Pulse magic+1% 19:19:19+50 ppm NAA+0.2% Borax .Foliar spray was taken at flowering stage pod filling stage. The results revealed that, foliar application of Pulse magic@1%+19:19:19@1%+ NAA@50 ppm + Borax@0.2% revealed that, the higher seed quality parameters viz: seedling vigour index I and II observed 2649 and 407, higher germination (92.67 %) respectively. The CCtc006 amplified an allele of size 230 bp in seed parent (ICPA 2039) but not its pollen parent (ICPR 2438). Further, the CCtc006 also amplified allele of size 220 bp in pollen parent (ICPR-2438) which restores the fertility in male sterile parent. Therefore, the presence of both the amplicon at 220 and 230 bp were noticed in $F_1$ hybrid, thus conforming hybridity of pigeonpea hybrid ICPH 2438.

## Introduction

Maintaining food and nutritional security for the increasing population of the world is a great challenge for us. Besides emphasizing on main crops and vegetables, various pulses also play an important role to satisfy the growing human food demands. Pulses are important source of protein particularly for the poor people and provides nutritional security. But pulses are mostly grown by the small and marginal farmers under resource constraints situation. The Pigeonpea [*Cajanus cajan* (L.) Mill sp.] is a member of the subtribe Phaselous, belongs to leguminaceae family and native to Africa. It is also known as tur or redgram. It is most drought tolerant among all pulses. It is deep rooted, $C_3$ and short day plant. It requires temperatures between 18-30 °C and sandy loam to clay loam soils are well suited.

Pigeonpea represents 6.22 million ha of world pulse's area and 4.74 million tonne of world's

pulse production. (Indiastat, 2018-19). In India pigeonpea occupied an area of 3.96 million ha with production of 2.56 million tonne. Pigeonpea is cultivated in larger part of the area is in states such as Maharashtra, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh and Tamil Nadu, which together hold 87.89 per cent and 86.10 per cent area and production, respectively.

The important challenge in sustainable hybrid production is managing the supply of adequate quantities of pure hybrid seeds of farmers. Like hybrids for any crop, a high level of genetic purity is essential in maintaining the necessary level of heterosis observed in the crops. Traditionally, breeders or seed companies carry Grow- Out Test (GOTs) on representative samples of the seed lot to assess the purity of hybrid seeds. The GOT involves growing plants to maturity and assessing several morphological and floral characteristics to determine the purity of the hybrid. As only one cycle of crop can be grown annually in pigeonpea, it will take almost a full cropping season to assess the purity of hybrid seeds by using conventional GOT. This, in turn, will result in major capital investment in storage the seeds for the next cropping season. Furthermore, GOT can be subjective, as several aspects of plant phenotype can be affected by environment conditions. Thus there is an urgent need for a precise and efficient assay in pigeonpea so that hybrid seeds produced in one season can be released for marketing and cultivation in the same season

The traditional Grow Out Test (GOT) is done to determine the seed genetic purity test based on morphological markers are time consuming and are environmental dependence. To overcome this disadvantage, the biochemical markers are being used in many crops. However, repeatability and accuracy of these results on biochemical markers are subject to question. This made a way for the use of DNA molecular markers particularly the co-dominant markers. The SSR markers are of great importance for rapid assessment of hybrid and parental line seed purity (Yashitola *et al.,* 2002)

## Materials and Methods

The experiment on effect of foliar spray of nutrients, growth regulators on seed yield and seed quality in hybrid pigeinpea [*Cajanus cajan* (L.) Millsp] was conducted at Seed Farm, College of Agriculture, Vijayapura, during *kharif* 2019-2020. The experiment was laid out in randomised block design. The experiment was laid out in randomised block design with 11 treatments. The treatments with foliar spray of namely control ($T_1$), 1% Pulse magic ($T_2$), 1% 19:19:19 ($T_3$), 0.2% Borax ($T_4$), 50 ppm NAA ($T_5$), 1% Pulse magic+1%19:19:19 ($T_6$), 1% Pulse magic+0.2% Borax ($T_7$), 1% Pulse magic+50 ppm NAA ($T_8$), 1% Pulse magic+1% 19:19:19+0.2% Borax ($T_9$), 1% Pulse magic+1% 19:19:19+50 ppm NAA ($T_{10}$), 1% Pulse magic+1% 19:19:19+50 ppm NAA+0.2% Borax ($T_{11}$) with 3 replications. Foliar spray was taken at flowering stage pod filling stage. The crop was cultivated by following the recommended package of practices for cultivation. Pigeonpea seed quality parameters like germination percentage, root length, shoot length, seedling vigour index, test weight, electric conductivity and protein content. The data obtained from a set of observations for each character will be tabulated and method of "Analysis of variance" as suggested by Fisher and Yates (1938). Pure seeds of these hybrid and their parental lines were harvested from Experiment I will be utilized for genetic purity assessment. These were procured from the pigeonpea breeding unit of ICRISAT, Patancheru, Andhra Pradesh, India.

**Pigeonpea hybrids and their parental lines used in the study**

| Hybrid | Silent features of hybrids | Seed parent | Pollen parent |
|---|---|---|---|
| **ICPH-2438** | Short duration with 33% higher yield compared to best cultivar Asha | ICPA-2039 | ICPR-2438 |

DNA was extracted using CTAB protocol. About 0.1 g of young leaf tissue from each sample was homogenized in liquid nitrogen and incubated at 600C for 30–45 min with 500 µl of CTAB buffer (1.0 M pH 8.0 Tris-HCl, 3 ml NaCl, 0.5 EDTA, 1% PVP-360). Then 500 µl of chloroform: isoamyl alcohol mixture (24:1) was added and blended thoroughly. After centrifugation (5 min, 13,000 rpm), supernatant layer was pipette out into a new eppendorf tube and an approximately equal volume of chilled ethanol was added. After storage at -20°C for 30–60 min, precipitated DNA was centrifuged, vacuum dried and finally stored in TE buffer.

## PCR amplification

25 SSR primer pairs were used in the study. The volume of the reaction mixture was 12 µl. It consists of 30 ng of template DNA, 1 × PCR buffer with 1.5 mM of MgCl2, 0.2 mM of dNTPs, 0.25 µM each of forward and reverse primers and 1U of Taq DNA polymerase. Thermal Cycler was used and programmed for 30 cycles of 94°C (3 min), 50-55°C (40 sec.), 72°C (40 sec.), then followed by final extension at 72°C for 10 min. PCR products (7.0-7.5 µl) were used for electrophoresis on six percent acryl amide gels stained with ethidium bromide at 100-120 V/cm for 100-200 min and photographed using documentation unit under UV light.

## Results and Discussion

In the present study, the seed germination percentage was significantly influenced by foliar spray of nutrients and growth regulators(Table 1). Among all the different foliar spray treatments $T_{11}$ (Pulse magic @ 1 % + 19:19:19 @ 1 % + NAA 50 ppm + Borax @ 0.2 %) significantly higher seedling vigour index I and II observed 2649 and 407 respectively, it is mainly due to higher germination (92.67 %), higher root and shoot length (18.67 cm and 15.08 cm), higher seedling length (37.75 cm), lower electric conductivity (0.59 $dSm^{-1}$) and higher seed protein (22.18 %) as compared to all the treatments was seen in Table 1 and control recorded with a lower seedling vigour index I and II 2047.7, 294.40 which contributes to lower germination percentage (84.00 %), lower root and shoot length (13.08 cm and 9.75 cm), lower seedling length ( 26.64 cm), higher electric conductivity (0.74 $dSm^{-1}$) and lower protein content (19.68 %). This is having a positive relation to seed development which is reflected through the size and test weight of seed and the micronutrients present in it. This in turn dependant on efficient synthesis, accumulation of food metabolites such as protein, carbohydrates and their translocation from source to the developing seed at greater ease. Seed with higher initial capital food reserves always showed higher and rapid germination which is also true in present study. Similarly, the micronutrients also participate in catalytic activity and breakdown of complex food source to simple form (glucose, amino acids and fatty acids). These in turn reflected on enhancing the rate of germination and elongation of root and shoot. Whereas higher reading for conductivity match the seed lots with inferior quality, and which is related to disruption of the membrane system, releasing higher rates of

compounds into the watery environment. NAA and boron increases membrane integrity via a close arrangement of lipid and protein. It also enhances bonding between cellulose fibres in the cell wall. Similar results found by Jadav *et al.,* (2019). similar results were reported by Kavitha *et al.,* (2002) in black gram as seeds hardened and pelleting and foliar spray with micronutrients with 40 g DAP + 100 mg 50 ppm NAA + 100 mg $FeSO_4$ + 250 mg ammonium molybdate per kg of seed, and Masuthi *et al.,* (2009) with Zinc + Boron + Arappu leaf powder + 19:19:19 in cowpea.

The CCtc006 amplified an allele of size 230 bp in seed parent (ICPA 2039) but not its pollen parent (ICPR 2438) depicted in plate 1. Further, the CCtc006 also amplified allele of size 220 bp in pollen parent (ICPR-2438) which restores the fertility in male sterile parent. Therefore, the presence of both the amplicon at 220 and 230 bp were noticed in $F_1$ hybrid, thus conforming hybridity of pigeonpea hybrid ICPH 2438 was seen in Plate.1.

The SSR markers identified both seed and pollen parent specific bands and are useful in genetic purity testing. These markers have an advantage of co-dominance inheritance, easy scoring of the alleles, reproducibility and accessibility to laboratories (Saxena *et al.,* 2010). The use of SSR markers for genetic purity testing has also been demonstrated in cotton (Ashok and Vilas, 2005).From the above discussion, it can be concluded that, pigeonpea hybrid could be distinguishable clearly from its parental lines using SSR markers. CCtc006 can be confidently used for identification of ICPH-2438.

**Table.1** List of primers used

| Sl. No. | Primer | Forward (F) / Reverse (R) Primer sequence 5'-3 | Ann. Temp. (Tm) |
|---|---|---|---|
| **1** | CcM0008F | CGGTGAAAAGGGTCAATGAG | 46.8 |
| | CcM0008R | CAAAATTAAAGCCTACTTATTTTACGA | |
| **2** | CcM0021F | TGAATGTTTTCCAGGATTTTACA | 46.8 |
| | CcM0021R | GCGCAAATATAAGAGCCCAG | |
| **3** | CcM0030F | GCAATATCAATTCAATGGTGGA | 48.0 |
| | CcM0030R | TGACAGATGCACTCTCTCGTTT | |
| **4** | CcM0039F | AGGAATAATGTTTGCTGCGG | 44.7 |
| | CcM0039R | TTGGTATGTGGAACGATTGC | |
| **5** | CcM0047F | TGTCTTTTGGATGAAAGTAGGGA | 46.8 |
| | CcM0047R | GTTGGGGATGGGAAGAGAAT | |
| **6** | CcM0051F | ACCTTTATTTTGAGCAGGAAAA | 44.2 |
| | CcM0051R | TGAATCATTTTCTGTTGAAGGG | |
| **7** | CcM0057F | CAATGTTGGCATAGGAACCA | 44.7 |
| | CcM0057R | GCTTAAAACTTGTGGGGCAA | |

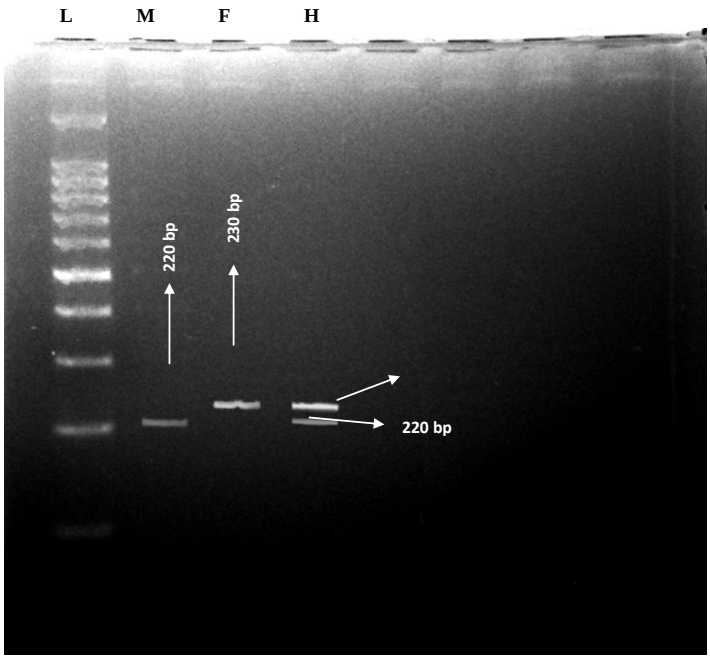
| Sl. No. | Primer | Forward (F) / Reverse (R) Primer sequence 5'-3 | Ann. Temp. (Tm) |
|---|---|---|---|
| **8** | CcM0080F | TGCATGTTGTATGTTGGTTGG | 45.5 |
| | CcM0080R | GTAATGCATCTCAATAATTTCAACA | |
| **9** | CcM0082F | TGTCAATGTCATGTTGGGCT | 46.8 |
| | CcM0082R | CATGCCATCTTCCTTTCTCC | |
| **10** | CcM0093F | TCATTGACCCCTCTGGAAAT | 44.7 |
| | CcM0093R | ACAATTGGAAAAATAAGTGAGTGAT | |
| **11** | CcM0095F | ATAATTAGTGGGCTGGGCCT | 46.8 |
| | CcM0095R | TGCCTCATAATCATGTTGCTTC | |
| **12** | Cctc006F | GCGCTAAGGGAAAACAAAAA | 44.7 |
| | Cctc006R | AACTCCCTTGTTGTCATATGGTG | |
| **13** | CcM0121F | AGAAATTGGAGGCTTGGTCA | 46.8 |
| | CcM0121R | GGTATAAGGCTCAAACCCGA | |
| **14** | CcM0126F | TGGTCCATGTTCCTCACTCA | 46.8 |
| | CcM0126R | CCAATGAAAATGAGAACCTTCA | |
| **15** | CcM0133F | GTTGTCCCATTTTGACCTCC | 46.8 |
| | CcM0133R | CCATAATCCAATCCAAATCCA | |
| **16** | CcM0134F | CTCTGCCCCGATGTCATATT | 46.8 |
| | CcM0134R | TTGGGATGTGAAGATGATGAA | |
| **17** | CcM0179F | GCAAAATTGCACTAAAATTTGTTT | 46.8 |
| | CcM0179R | CCATCTTCGCCTGTCGTATT | |
| **18** | CcM0183F | GCCCATTTTGTCATCCCTAA | 44.7 |
| | CcM0183R | TTCAACAGTTGGATCGTTCA | |
| **19** | CcM0185F | TTGATCATGACTTATGCCTTTGA | 46.8 |
| | CcM0185R | GGCTTGCTTTGAGTTCCTTG | |
| **20** | CcM0193F | TAAATCACCACCCTTGAGGC | 46.8 |
| | CcM0193R | TGCAAAAACACATCCTGGAA | |
| **21** | CcM0195F | CAACAATAAAGCATAAACCACCA | 48.5 |
| | CcM0195R | TGACGTAGATTGGGTAGTTAGGA | |
| **22** | CcM0207F | TTTTGGCGGTCATTTTAACC | 43.5 |
| | CcM0207R | TTAGTCGGGAGCAACATGA | |
| **23** | CcM0208F | GCATCTAAATACAATTAATATTGTGGG | 48.8 |
| | CcM0208R | ATAGGGTGGATCTCTGGTGC | |
| **24** | CcM0246F | ATGGAGCCAAAGTGTCCAAG | 46.8 |
| | CcM0246R | ATGAAAAGCAACTACGCGCT | |
| **25** | CcM0248F | TGGAATTTGACTCATTTAGAATAGGA | 47.4 |
| | CcM0248R | CCCACAGACAGCATATCAACA | |

**Table.2** Effect of different sources of nutrients and growth regulator on seed quality parameters in pigeonpea

| Treatments | Germination percentage (%) | Shoot length (cm) | Root length (cm) | Seedling length (cm) | Vigour Index I | Vigour Index II | Electrical conductivity (dSm-1) | Protein percentage (%) |
|---|---|---|---|---|---|---|---|---|
| **$T_1$** | 84.00 | 9.75 | 13.08 | 26.64 | 2047.70 | 294.40 | 0.74 | 19.68 |
| **$T_2$** | 87.00 | 10.03 | 15.33 | 28.83 | 2180.00 | 338.00 | 0.68 | 20.95 |
| **$T_3$** | 85.67 | 10.17 | 15.83 | 32.17 | 2107.00 | 316.20 | 0.64 | 20.92 |
| **$T_4$** | 87.00 | 14.02 | 15.25 | 32.42 | 2138.00 | 323.00 | 0.626 | 21.49 |
| **$T_5$** | 88.73 | 13.12 | 14.42 | 31.75 | 2146.00 | 339.60 | 0.65 | 21.93 |
| **$T_6$** | 86.33 | 14.17 | 16.08 | 32.83 | 2300.00 | 344.50 | 0.653 | 21.92 |
| **$T_7$** | 88.50 | 13.17 | 15.92 | 32.42 | 2444.00 | 340.20 | 0.64 | 21.69 |
| **$T_8$** | 88.00 | 13.08 | 16.33 | 32.58 | 2395.00 | 342.40 | 0.63 | 21.30 |
| **$T_9$** | 89.00 | 13.27 | 18.33 | 34.08 | 2444.00 | 343.10 | 0.626 | 21.65 |
| **$T_{10}$** | 89.50 | 14.92 | 18.08 | 36.83 | 2605.00 | 350.30 | 0.62 | 21.97 |
| **$T_{11}$** | 92.67 | 15.08 | 18.67 | 37.75 | 2649.00 | 407.00 | 0.59 | 22.18 |
| **Mean** | **87.85** | **12.80** | **16.14** | **32.77** | **2308.00** | **339.00** | **0.646** | **21.43** |
| **S.Em. ±** | 0.65 | 0.249 | 0.463 | 0.377 | 205.28 | 12.91 | 0.053 | 0.412 |
| **C.D. at 1 %** | 1.87 | 0.730 | 1.847 | 1.503 | 818.416 | 37.16 | 0.154 | 1.643 |

**$T_1$ - Control**
**$T_2$ - Pulse magic @ 1 %**
**$T_3$ - 19:19:19 @ 1 %**
$T_4$ - Borax @ 0.2 %
$T_5$ - NAA @ 50 ppm
$T_6$ - Pulse magic @ 1 % + 19:19:19 @ 1 %
$T_7$ - Pulse magic @ 1 % + Borax @ 0.2 %
$T_8$ - Pulse magic @ 1 % + NAA 50 ppm
$T_9$ - Pulse magic @ 1 % + 19:19:19 @ 1 % + Borax @ 0.2 %
$T_{10}$ - Pulse magic @ 1 % + 19:19:19 @ 1 % + NAA 50 ppm
$T_{11}$ - Pulse magic @ 1 % + 19:19:19 @ 1 % + NAA 50 ppm + Borax @ 0.2 %

**Plate.1** SSR marker Cctc006 profile confirming hybridity of pigeonpea hybrid ICPH 2438 obtained on Ethidium bromide stained on agarose gel (3.5 %)




Note: L – Ladder M - Male F - Female H – Hybrid

In conclusion seed quality parameters influenced significantly due to foliar feeding of nutrients and growth regulators sources. The significantly higher seed quality parameters were seen in foliar spray of Pulse magic @ 1 %+ 19:19:19 @ 1 % + NAA 50 ppm+ Borax @ 0.2 % recorded higher seed quality parameters followed by combined foliar spray nutrients and growth regulators, while lowest seed quality parameters was recorded in to control.

The utility of SSR markers in detecting genetic purity of pigeonpea hybrids ICPH 2438 was investigated the CCtc006 amplified an allele of size 230 bp in seed parent (ICPA 2039) but not its pollen parent (ICPR 2438). Further, the CCtc006 also amplified allele of size 220 bp in pollen parent (ICPR-2438) which restores the fertility in male sterile parent. Therefore, the presence of both the amplicon at 220 and 230 bp were noticed in $F_1$ hybrid, thus conforming hybridity of pigeonpea hybrid ICPH 2438.

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