SUMMARY AND CONCLUSIONS
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Oil seeds, their oil and high protein meal products are the most valuable commodity on the world trade. Niger \([Guizotia abyssinica \text{(L.f.)}}\) Cass.] plant belonging to family Asteraceae, native to tropical Africa, has been regarded as a minor oilseed crop in India. Niger is mainly grown in India and Ethiopia. Recently it is gaining importance as it is exported to U.S.A., U.K. and Canada as birdseed. Niger is a completely out crossing species with sporophytic incompatibility mechanism. Self-incompatibility of niger hinders the production of homozygous lines, ultimately crop improvement also. One of the main applications of anther culture has been to produce diploid homozygous pure lines in a single generation, thus saving many generations of back crossing to reach homozygosity by traditional means or in crops where self-pollination is not possible. So, the present work was undertaken to develop a protocol to induce haploids by anther and microspore culture.

Anthers of niger cvs. Ootacamund and JNC-6 were cultured on B5 medium containing 0.2 M sucrose and auxins (IAA, IBA, 2,4-D and NAA). Among the four auxins tested at different levels of concentrations (0.5 \(\mu\text{M}, 1 \mu\text{M}, 2 \mu\text{M}, 5 \mu\text{M}, 10 \mu\text{M} \text{ and} 20 \mu\text{M})\), 2,4-D induced callus mediated embryogenesis at 2 \(\mu\text{M}, 5 \mu\text{M} \text{ and} 10 \mu\text{M} \) supplemented B5 medium. Highest percentage of response (23.33 and 13.8) and (9.33 and 3.66) number of embryos per treatment were observed from anthers of cvs. Ootacamund and JNC-6 cultured on B5 medium supplemented with 10 \(\mu\text{M}\) 2,4-D respectively. Similarly in NAA supplemented medium, highest percentage of response (13.33 and 7.2) and highest mean number of embryos (5.0 and 2.33) was recorded from the two cultivars Ootacamund and JNC-6.
respectively. No embryogenesis was observed from anthers cultured on IAA, BA, KN, TDZ, ADE and 2-ip supplemented individually to B5 medium.

2,4-D and NAA (5 μM and 10 μM) in combination with cytokinins (BA/KN/TDZ/ADE/2-ip at concentrations 0.5 μM, 1 μM and 2 μM) induced high percent of response than the 2,4-D and NAA supplemented alone medium in both the cultivars. Anthers of cvs. Ootacamund and JNC-6 cultured on combination of 10 μM 2,4-D and 2 μM KN yielded 53.66 and 51.66 mean number of embryos per treatment respectively. Similarly, from combination of NAA (5 μM and 10 μM) and cytokinins (BA/KN/TDZ/ADE/2-ip at concentrations 0.5 μM, 1 μM and 2 μM), the best results (41.33 and 38.33 number of embryos per treatment respectively) were obtained from anthers cultured on medium containing 10 μM NAA + 2 μM KN and 0.2 M sucrose.

In the present study, external stress, particularly cold pretreatment significantly increased the embryo induction. For both the cultivars, cold temperature pretreatment at 4 °C for 3 days proved to be favourable in induction of more responding anthers. Induction of number of embryos was more than the control in capitula cold pretreated for 4, 5 and 6 days but gradually embryogenesis decreased and complete inhibition was observed from anthers of 9 and 10 days cold pretreated capitula. Heat pretreatment at 32 °C was not effective as cold pretreatment.

Among the different carbohydrate sources supplemented to B5 medium, sucrose proved to be the best in induction of embryogenesis. The highest number of 46.66 percent of responding anthers of niger cv. Ootacamund produced 57.66 mean numbers of embryos and 2.05 mean
number of embryos per responding anthers on medium containing 0.2 M sucrose. Anthers of cv. JNC-6 cultured on the media containing 0.2 M sucrose, showed greatest number of responding anthers (42.77), mean number of embryos (54.33) at the rate of 2.11 embryos per responding anther. Embryogenesis was not observed from anthers cultured on 0.05 M sucrose, 0.05 M and 0.1 M glucose, 0.05 M fructose and 0.05 M maltose. 0.4 M glucose induced highest number of 19.66 embryos per treatment in cv. Ootacamund anthers whereas in JNC-6, 0.35 M glucose induced highest number of 16.33 embryos per treatment. Anthers of cvs. Ootacamund and JNC-6 cultured on 0.4 M fructose induced 17.33 and 12.00 embryos per treatment respectively. Embryogenesis was observed in only few concentrations (0.1 M, 0.15 M, 0.2 M and 0.25 M) of maltose tested in both the cultivars. Highest embryogenesis from anthers of cvs. Ootacamund and JNC-6, observed was 13.66 and 10.66 per treatment respectively in medium supplemented with 0.15 M maltose.

To enhance the frequency of embryogenesis B5 medium was supplemented with amino acids (arginine, asparagine, cysteine, glutamine, glycine and proline) individually, highest embryogenesis of 63.33 and 61.66 embryos per treatment were observed from anthers of both the cultivars, cultured on B5 medium supplemented with 2 mM proline +10 μM 2,4-D + 2 μM KN and 0.2 M sucrose. This was followed by a combination of all the six amino acids in concentration 1mM, inducing 61.33 and 60.33 embryos per treatment from anther of cvs. Ootacamund and JNC-6 cultured on B5 medium supplemented with 10 μM 2,4-D + 2 μM KN and 0.2 M sucrose.

Addition of polyamines (putrescine and spermidine) in seven different concentrations to B5 medium supplemented with 10 μM 2,4-D + 2 μM KN
and 0.2 M sucrose induced embryogenesis higher than the control. Optimum number of embryos per treatment (57.00) was observed from anthers of niger cv. Ootacamund cultured on 100 μM putrescine and 56.66 embryos per treatment from anthers of the same cultivar cultured on 200 μM spermidine. In niger cv. JNC-6 also, highest embryos per treatment (52.66 and 51.33) were observed from anthers cultured on 100 μM putrescine and 200 μM spermidine added to B5 medium along with 10 μM 2,4-D + 2 μM KN and 0.2 M sucrose.

100 μM silver nitrate added to B5 medium with 10 μM 2,4-D + 2 μM KN and 0.2 M sucrose induced highest number of 54.33 and 51.66 embryos respectively from the anthers of niger cvs. Ootacamund and JNC-6 respectively.

Addition of 1 μM jasmonic acid to B5 medium containing 10 μM 2,4-D + 2 μM KN and 0.2 M sucrose improved embryogenesis (45.66) from anthers of niger cv. Ootacamund more than the control (39.66). Anthers of cv. JNC-6, cultured on medium supplemented with 1 μM jasmonic acid induced 40.33 embryos per treatment, which is slightly higher than the control (38.33).

Different media such as B5, MS, NN, N6 and LS medium with 10 μM 2,4-D + 2 μM KN and 0.2 M sucrose tested for induction of embryogenesis, highest percentage of responding anthers (44.44 %) induced 60.66 mean number of embryos as well as 2.27 embryos per responding anther on B5 medium. This was followed by LS medium with 31.66 % of response and 30.66 mean numbers of embryos. Other three media were not much significant in inducing embryogenesis.
Among the ten selected genotypes of niger tested for embryogensis, cultivar Ootacamund gave the best results of 52.33 embryos per treatment, followed by JNC-6 (45.66) and IGP (41.66).

Out of the three embryo differentiation media used, in ED-I medium, optimal number of embryo differentiation was observed at 0.5 μM BA with 16.66 and 15.66 mean number of embryos differentiation from cultivars Ootacamund and JNC-6 respectively. In ED-II medium, 2 μM proline was effective in bringing out 16.33 and 14.66 embryo differentiation per treatment; this was followed by medium containing 2 μM glutamine wherein 15.33 and 13.33 embryos differentiated from both the cultivars respectively. In ED-III medium, in medium with 2 μM putrescine, 5.00 and 4.00 embryo differentiation and in medium containing 2 μM spermidine, 4.66 and 4.33 embryo differentiation was recorded respectively.

For maturation of embryos, ABA treatment was must. Among all the concentrations of ABA supplemented to B5 medium with 0.09 M sucrose, 10 μM ABA was more effective for both the cultivars in bringing out the highest number of embryo maturation. Embryos originated from different induction medium showed different frequency of embryos maturation.

Germination of mature embryos was achieved on B5 basal medium supplemented with 0.09 M sucrose. Germination of embryos was strongly dependent on the embryo induction medium.

Histological study showed both microspore and anther wall callusing. Thirty-four plantlets survived out of sixty transplanted plants. Among those survived plantlets, root tip cytological studies showed twelve plants with the haploid chromosome number and the remaining were diploids. Flow
cytometric analysis of nuclei prepared from the anther culture derived plantlet showed a peak with lower DNA content than the standard diploid.

Though initial symmetrical division could be induced in isolated microspore culture, further haploid callus or embryo formation could not be achieved.

Based on the results obtained in the present study, following conclusion could be drawn:

- Among the four auxins, IAA, IBA, 2,4-D and NAA added to B5 medium with 0.2 M sucrose for induction of embryogenesis, 2,4-D induced more number of embryos in both the cultivars. NAA induced embryogenesis in only two concentrations.
- Auxins (2,4-D and NAA) in combination with cytokinins (BA/KN/TDZ/ADE/2-ip) bring out more number of embryos per treatment than the medium containing auxin alone. Combination of 10 μM 2,4-D + 2 μM KN and 10 μM NAA + 2 μM KN with 0.2 M sucrose gave the best results.
- Cold temperature pretreatment at 4 °C for 3 days proved to be the best in induction of more responding anthers in both the cultivars.
- Medium containing 0.2 M sucrose induced more number of embryos per treatment and also, highest percent of embryo germination.
- Amino acid proline, at all the concentrations used in this study had an overall beneficial effect on the cultures and yielded more number of embryos per treatment. Combination of six amino acids at 1mM was also significantly effective in inducing embryogenesis.
- Polyamines, putrescine and spermidine enhance the embryo yield per treatment.
Silver nitrate up to 500 µM increased the embryogenesis in both the cultivars.

Jasmonic acid was not much effective in bringing out embryogenesis.

B5 medium proved to be the best medium for induction of embryogenesis.

Among ten genotypes tested, cvs. Ootacamund and JNC-6 were the best responding varieties.

B5 medium supplemented with 0.09 M sucrose and 0.05 µM BA is the best medium for embryo differentiation.

For embryo maturation B5 medium supplemented with 0.09 M sucrose and 10 µM ABA is the best.

Anther culture of niger yields both haploid and diploid plants.

Isolated microspore culture in niger was not successful to the full extent of bringing out haploid embryos and plantlet.

Present study gives an improved protocol for the production of haploids through anther culture. Compared to earlier studies, this protocol clearly gives an account of induction, differentiation, maturation and germination medium for the anther culture studies in niger. Also, enhanced embryogenesis could be achieved using different carbohydrate sources, amino acids, polyamines, silver nitrate and jasmonic acid.