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SUMMARY AND CONCLUSION

The symbiotic interaction between rhizobia and legume roots is characterized by a high degree of specificity. Nod factor, a substituted lipochitooligosaccharide synthesized as a result of the expression of rhizobial *nod* genes, is a key factor in the initiation of nodule development. Rhizobial *nod* gene expression is mediated by the transcriptional activator Nod D, which interacts with plant-secreted flavonoids and non-flavonoid inducers. These interactions also can be, a highly specific. This indicated that the *Rhizobium*-legume interactions have been reported to be very specific in nature.

Two varieties of cowpea were gamma irradiated as a one method to create genetic variation resulting in new varieties with better characteristics in nodulation and nitrogen fixation processes. Conjugation is the second method used in this study, a cell contact-dependent DNA transfer mechanism, which has served as elegant tool in the development of genetic engineering technology. The possibility of horizontal gene transfer to other rhizobia, revealed that it is necessary, in view of possibility of deliberate release of a variety of recombinant rhizobia into the environment for such agricultural purposes as improving nitrogen fixation.

The detection of gene transfer in the environment also depends on the selective advantage (or disadvantage) that the gene under consideration confers upon the recipient cell. Nitrogen fixation by symbiotic rhizobia is of enormous economic importance, being responsible for the global fixation of about 50 million metric tons of nitrogen each year. This is very important because nitrogen is an essential plant nutrient, that is most commonly deficient is soils, contributing to reduced agricultural yields throughout the world. The improving process of nitrogen fixation is becoming more important for not only reducing energy costs, but also in seeking more sustainable agricultural production and reduced environmental pollution. This leading that nitrogen fixing microorganisms could therefore be an important component of sustainable agricultural systems.

The main objective of this study was to explore and discuss the possibilities for enhancing N_2 fixation by working on the plant host and the microbial symbiont that illustrate best practices and experiences for enhancing biological nitrogen fixation. The genetic contribution of the cowpea plants to establishment of a successful N_2-fixing symbiosis in the legume-*Rhizobium* system has been clearly demonstrated through genetic variations induced in macro- and micro-symbiont using gamma irradiation and horizontal gene transfer, respectively.

Cowpea (*Vigna unguiculata* L.) was used in this study because it is lower nodulating by rhizobia and is an important grain legume crop, it is essentially cultivated for fresh or dried seeds and it plays a considerable role in the nutritional and economical balances of these populations. Cowpea is consumed in many forms; the young leaves, green pods, and green seeds are used as vegetables; dry seeds are used in various food preparations, and the haulms are fed to livestock as nutritious supplement to cereal fodder. In West and Central Africa, cowpea is of major importance to the livelihoods of millions of people providing nourishment and an opportunity to generate income. It is a major source of protein, minerals and vitamins in daily diets, it positively impacts on the health of women and children. The addition of even small amount of cowpea ensures the nutritional balance of the diet and enhances the protein quality by the synergistic effect of high protein and high lysine from cowpea and high methionine and high energy from the cereals. This nutritious and balanced food ensures good health and enables the body to resist infectious diseases and slow down their development.

Cowpea rhizobia were first classified in an heterogeneous group of slow-growing rhizobia nodulating promiscuous tropical and subtropical legume species known as cowpea cross inoculation group. They were later transferred to the genus *Bradyrhizobium*, an indigenous population of *Bradyrhizobium* nodulating cowpea cultivars under favourable and water-deficient conditions. It appears that cowpea can be nodulated by at least four different *Bradyrhizobium* strains belonging to three genospecies. Advanced generation lines M_3 and M_4 generations derived from radiation-treated two cowpea cultivars were evaluated for their performance and response to the resulting transconjugants of *Rhizobium*.

The results obtained from this study are summarized as follows:-

1. The colorimetric assay used for determining indole compounds revealed higher amounts of the phytohormone was produced from tryptophan above the mid-parents in two out of six transconjugants resulted from the cross between P_1 x P_3.
2. Only one out of three transconjugants resulted from the cross between P_1 x P_2 accumulated the phytohormone above the mid-parents in the presence of lactic acid.
3. All transconjugants resulted from the cross between P_2 x P_3 accumulated the phytohormone above their mid-parents with the amounts ranging from 1.47 to 2.30 μg/ml in the presence of lactic acid.
4. Two triparental transconjugants resulted from the cross between P_4 x DPM-Tr_2 produced high amounts of indole compounds over their mid-parents from tryptophan and trypton.
5. One out of two triparental transconjugants resulted from the cross between P_4 x DPM-Tr_2 representative IAA from lactic acid over their mid-parents.
6. Diparental transconjugant-Tr_1 resulted a significant increase in the number of nodules on the root system of unirradiated v_1-variety in M_3 and M_4 generatrions above the mid-parents and the plants fertilized with recommended full dose of N.

7. Significant number of nodules were developed on the root system of v_2-variety in M_4 generation treated with 20 krad in response to inoculation with the parental strains (P_2 and P_3) and also in response to inoculation with triparental transconjugants (Tr_4 and Tr_5), this above that develop on the plants fertilized with recommended dose of N.
8. The results revealed the success of rhizobial strains and their recombinants to colonize and infect roots of cowpea, because of significant dry weight of nodules per plant can be obtained in v_1-variety treated with 20 krad in M_4 generation inoculated with the parental strain (P_3), above that on the plants fertilized with recommended dose of N.
9. Both M_3 and M_4 generations of v_2-variety treated with 10 krad had significantly higher average weight of nodule in response to most inoculum of parental strains and their recombinants, indicated that the dose of 10 krad may affect on the loci governing nodule weight.
10. Significant increase in nodulation index was also shown in v_2-variety of unirradiated plants (M_4) and that treated with 10 krad (M_4) and 20 krad (M_3) in response to the infection with some rhizobial strains and their recombinant.
11. Shoot dry weight (DW) of unirradiated plants of v_1-variety in M_3 generation was significantly increase above the plants fertilized with recommended dose of N in response to inoculation with the parental strain (P_1) and new recombinant isolates (DPM-Tr_1, DPM-Tr_2, TPM-Tr_4 and TPM-Tr_5).
12. Plants inoculated with bacterial transconjugants were promoted greater growth rate above that fertilized with recommended dose of N, because of phytohormones produced by rhizobial strains.

13. No significant increase in the number of branches and plant height was shown above the mid-parents and the plants fertilized with recommended dose of N, in response to inoculation with any of transconjugants.

14. Better leaf area can be obtained above that in the full dose in M_3 generation of v_2-variety treated with 10 krad, in response to inoculation with the parental strain (P_3) and new recombinant isolates (DPM-Tr_1, DPM-Tr_2, DPM-Tr_3 and TPM-Tr_5). The same trend was also shown in M_4 of v_2-variety treated with 20 krad in response to inoculation with the parental strains P_1 and P_2.

15. Some rhizobial strains; P_2-inoculated M_3 and M_4, P_3-inoculated M_4 generation treated with 30 krad and P_3-inoculated unirradiated plants can form significant concentration of chlorophyll b in v_1-variety above that formed in plants fertilized with recommended dose of nitrogen.

16. Many recombinants induced significant concentration of chlorophyll b above their mid-parents in v_1-variety, indicated that this variety responded well to all new recombinants of rhizobia at different doses of gamma irradiation.

17. Total chlorophyll formation in v_1-variety inoculated with di-parental transconjugants (DPM-Tr_2 and DPM-Tr_3) at all doses of gamma irradiation was significantly increase above that in the plants fertilized with recommended full dose and the mid-parents, with the exception at 30 krad if compared with the mid-parents.

18. Great chlorophyll formation above that at the full dose was also obtained at all doses of gamma irradiated-v_2-variety in response to inoculation with TPM-Tr_4 and TPM-Tr_5, except for the plants treated with 10 krad and inoculated with TPM-Tr_5.

19. The importance of tri-parental transconjugants induced in this study was due to harbouring DNA from *Pseudomonas putida*, which may played a dual role by reducing disease incidence and promoting plant growth, resulted increased biomass and yield.

20. Significant increase was resulted in fresh weight of pods developed per plant above the mid-parents in M_3 generation of v_1-variety at doses zero and 10 krad, in response to inoculation with di-parental transconjugant, DPM-Tr_2. While, the same trend was also achieved above the full dose in M_3 generation at 10 krad in response to inoculation with DPM-Tr_2, DPM-Tr_3, TPM-Tr_4, and TPM-Tr_5.

21. Only one recombinant (DPM-Tr_2) exhibiting significant increase in fresh pods above the mid-parents and the recommended dose of N in M_4 generation of v_2-variety treated with 10 krad. Some inoculants affect to significantly increase the weight of fresh pods per plant.

22. M_3 generation resulted from v_1-variety treated with 20 krad appeared significant increase in 100-seeds weight above that in the plants fertilized with recommended dose of nitrogen, in response to most inoculants of *Rhizobium* strains and their recombinants.

23. Unirradiated v_2-variety exhibited significant increase in 100- seeds weight in M_3 generation above that in the plants fertilized with recommended dose of nitrogen, in response to all inoculants with the parental strains and their recombinants.

24. The parental strain P_2 and some of transconjugants (DPM-Tr_2, TPM-Tr_4 and TPM-Tr_5) significantly improved shoot nitrogen content of v_1-variety at all doses of gamma irradiated plants above the plants fertilized with recommended dose of nitrogen.

25. The highest nitrogen content was appeared in the shoots of v_1-variety at all doses of gamma irradiation in response to inoculation with diparental transconjugant (DPM-Tr_2). However, v_2-variety had the lowest nitrogen content in relation to the plants fertilized with recommended dose of nitrogen and to the mid-parents of rhizobial transconjugants.

26. Significant concentration in the content of shoot-nitrogen in M_4 generation of v_2-variety treated with 10 and 30 krad above the mid-parents was obtained in response to inoculation with di-parental transconjugant (DPM-Tr_2). Although, the nitrogen contents in the shoots was significantly increased in v_1-variety than in v_2 in response to inoculation with rhizobial transconjugants, above the mid-parents and the plants fertilized with recommended dose of nitrogen.

27. The genetic variability of seeds -protein content appeared that v_2-variety treated with 10 krad had significant increase in protein content above that in the plants fertilized with recommended dose of N among M_3 and M_4 generations, in response to inoculation with the parental strains and most of their transconjugants. The same trend was also shown in M_4 generation of v_1-variety treated with 20 and 30 krad above the plants fertilized with recommended dose of nitrogen, in response to inoculation with di-parental transconjugants.

28. Insufficient transport of nitrogen fixed from the shoots to the seeds is related to high shoot nitrogen content as shown in v_1-variety. Therefore, v_2-variety had lower shoot nitrogen content because of sufficient transport of nitrogen to the seeds leading to high protein content in the grains.

29. Nodules DW and shoot DW per plant of v_1-variety was more effective in response to all sources of variation (inoculation with rhizobial strains, doses of gamma rays and the interaction between both of them).

30. Plant height of v_1-variety was more effective by doses of gamma rays, however nodulation parameters, shoot DW and shoot / root ratio were more effective by rhizobial inoculation. Inoculation is a major source of variations in most nodulation parameters and growth rate traits.
31. The interaction between biofertilization and gamma irradiation appeared insignificant effect in v_1-variety for all the parameters of yield components.
32. Shoot / root ratio of v_1-variety was mainly affected by biofertilization, while plant height was mainly affected by doses of gamma irradiation.
33. Both doses of gamma irradiation and biofertilization were affected on the performance of most traits of nodulation and growth rate parameters of v_1-variety.
34. The interaction between condition x doses in v_1-variety appeared significant effect on the number of nodules developed per plant, nodule DW / plant, average weight of nodule, shoot and root dry weight.
35. Significant effect of treatment on shoot / root ratio of v_2-variety in M_3 generation was mainly due to the effect of biofertilization, doses of gamma rays and the interaction between both of them.
36. The number of nodules developed per plant in v_2-variety was more effective with all sources of variations, however, shoot DW was also more effective with all sources of variations, except for the doses of gamma rays, which shown insignificant effect on this trait.
37. The number of pods developed per plant, average weight of pods / plant and 100-seed weight in v_2-variety were more effective with all sources of variations, which included the effect of biofertilization, doses of gamma rays and the interaction between both of them, while the number of branches / plant was more affected by the doses of gamma rays.

38. Great part of differences was mainly due to the direct effect of radiation and rhizobial inoculation, while the interaction between these two sources of variation showed significant effect on one parameter alone (100- seeds weight) of v_2-variety.

39. Interaction mean squares in v_2-variety between doses and biofertilization appeared significant effect on leaf area / plant (M_3), chlorophyll a (M_3 and M_4), chlorophyll b (M_3), total chlorophyll (M_3 and M_4), seed protein content (M_4), shoot N content (M_4) and pollen abortion (M_3 and M_4).

40. All biochemical traits studied were more affected by biofertilization than the doses of gamma rays and the interaction between biofertilization x doses. This indicated that the significance of treatments was mainly due to inoculation and particularly to gamma irradiation and the interaction between both of them.

41. The interaction between doses x biofertilization revealed significant effect in v_2-variety on leaf area / plant (M_3 and M_4), total chlorophyll (M_3 and M_4), seed protein content (M_4), pollen stained and unstained (M_3 and M_4).

42. The doses of gamma rays achieved significant effect on all biochemical parameters in v_2-variety, except for on chlorophyll b and nitrogen percent of the seeds. However, total chlorophyll pigments were mainly affected by biofertilization and the doses of gamma rays.

43. Biofertilization can improving biochemical quality of cowpea by reducing the need for applied nitrogen fertilizer and promotion plant growth.

44. Pollen abortion appeared a dose-response of gamma irradiation, while the dose of 30 krad showed pollen abortion percentage greater than 49%.