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COMPARATIVE STUDY TO INDUCTION OF HAPLOID PLANTS BY *"IN VITRO"* ANTHER AND OVARIES CULTURE OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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INTRODUCTION

There have been obtained experimentally to many culture species haploids plants and isogenic lines that served to obtained new varieties superior to their "parents". (3). However, in the reference materials, there have been signaled to tomatoes 3 types of obtaining of haploid callus and only one case (GRENHOFF and DOY, 1972) of regeneration of haploids plants by androgenesis. These plants were, most of them, abnormal in their morphology of the leaves and didn't form flower.(1).

Viewing the necessity of obtaining haploids plants in order to create and preserve isogenic lines, an extremely valuable material for varieties and hybrids of great economical value (2), we intend to try with tomatoes the identification of genotypes able to be handled "in vitro" both through androgenesis (the way that other specialists tried with unimportant results) and through gynogenesis, that is not signaled to tomatoes in reference materials, but it favored to many species the obtaining of haploid plants and isogenic lines whenever androgenesis failed in giving the expected results.(1,3).

MATERIAL AND METHOD

The biological material used in our research was represented by 15 tomato diploid genotypes (Lycopersicon esculentum L.): L_1, L_2, L_3, L_4, L_5, L_6, L_7, L_8, W_1, T_1, T_2, T_5, T_7, LS, LS – 2407, from which floral buds were harvested, buds with anthers comprising pollen in the stage of uninucleated microspores and ovaries with mature embryo sacs.

The sterilization of the floral buds was done by immersion in mercuric chloride 0,1% for ten minutes followed by repeated washing with sterile distilled water.

For induction of androgenesis and gynogenesis there were used 8 hormonal variants derived from the basic medium MS (Murashige – Skoog, 1962). The culture were incubated at dark, at 27 ^{0}C until the callus was completely formed. The callus was passed on a differentiation medium, achieved in 5 variants derived from MS (1962), in photoperiod conditions for 16 hours (3000 lx).

The number and structure of chromosome were established in the root meristems of the regenerated plants. The processing of the material was done according to the Feulgen classical method.

RESULTS AND DISSCUSIONS

After 4 weeks of culture, all the anthers of the genotypes used, except for the genotypes LS and LS – 2403 callused on the culture variants A_2, G_2, G_3 and G_4. The ovaries callused on the some variants, but only 9 genotypes of the 15 tested.

On the differentiation mediums, in photoperiod conditions, the androgynous callus generated numerous meristematic regions that evolved in very strong roots callusing when they came into contact with the medium, especially on variants V_1 and V_3.

When maintained on their induction mediums and at light it was observed that, in the case of L_3, L_6 and L_7 genotypes on the induction variants V_2, the callus formed embryoids that evolved in embryos and fully grown plants with roots. These plants were passed in tubes, on the MS medium without hormones or with 0,5 mg|l NAA. The plants had a normal evolution on the MS medium without hormones. On the medium with NAA, as the roots were forming, they callused just the same as the leaves that came into contact with the medium. After three weeks of culture into tubes, the plants of pollinic origin were passed in a hydroponics medium, so they could adapt to septic condition, than in pot and greenhouse.

The callus of gynogenetic origin had an evolution somehow different, as on V_2, V_4 and V_5 differentiation medium, it generated embryos and fully formed plants. The genotypes with a gynogenetic answered were marked L_2, L_5, L_6, L_7 and W_1. Out of the 22 plants obtained from ovaries were albinotic (40%), 9 had an abnormal evolution and only 5 of them had a normal evolution.(fig.4).

The preliminary cytogenetic stage done in the andro- and gynogenetic plants, proved a high frequency of the haploid (67%), compared with the regenerated diploid plants (33%) and the absence of other ploidy degrees.

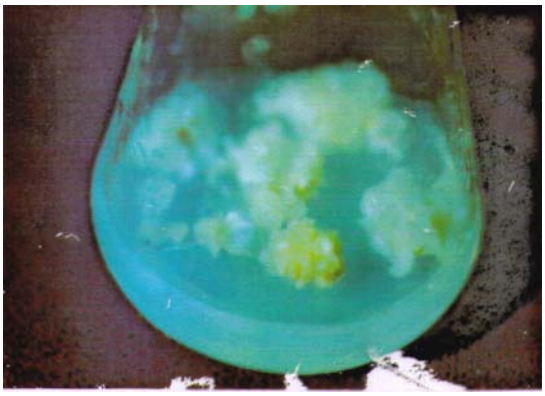

Fig. 1. Callus with gynogenetical origin

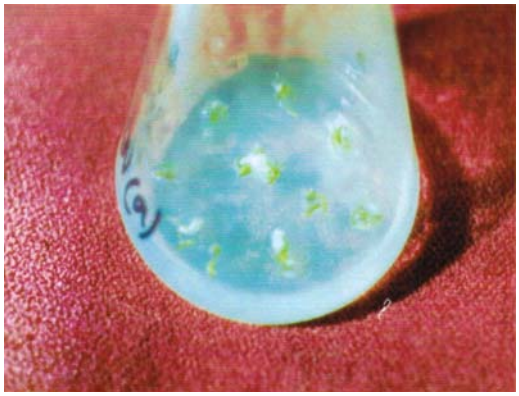

Fig. 2. Callus with androgenetical origin

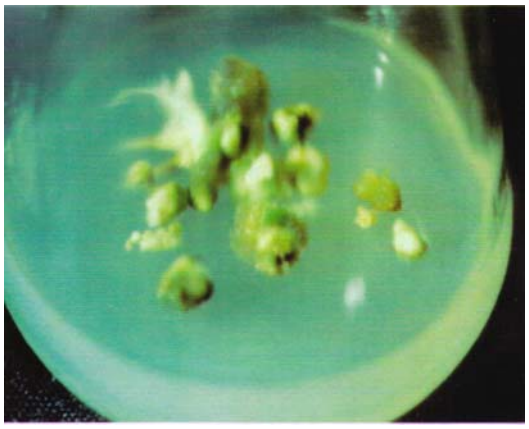

Fig. 3. Embryos and roots from ovaries

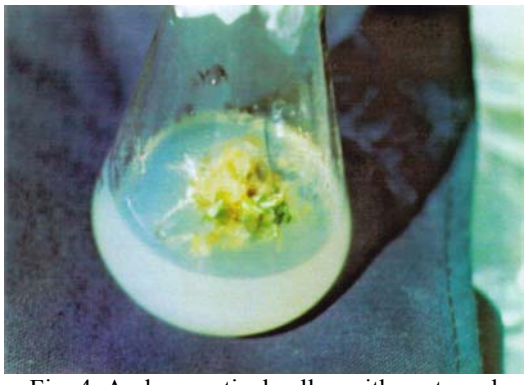

Fig. 4. Androgenetical callus with roots and ambryos

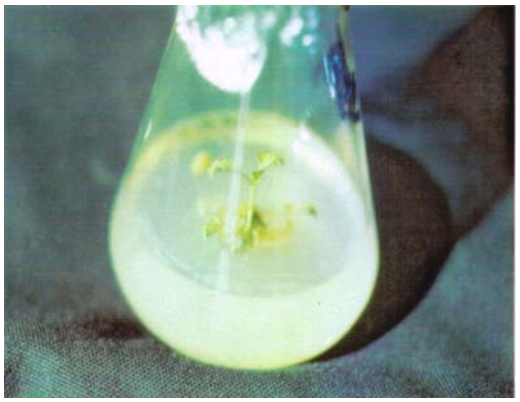

Fig. 5. Fully grown plant from ovaries

CONCLUSIONS

We therefore can say that it is possible to obtained cultivated tomatoes haploid plants through experimental androgenesis and gynogenesis. The success of this "in vitro" types depends on both genotype and culture conditions.

The best phytohormonal combination for the induction of embryogenic callus was the variants characterized by an equal proportion (1:1) between auxine and cytokinine (A_2, G_2, G_4) or a raised quantity of auxine as compared with cytokinine (G_3).

An equal quantity of auxine and cytokinine (variant A_2) allowed, in the case of pollinic callus, it's evolution to embryos and plants. As for the gynogenetic callus, it evolved in embryos and plants on variants V_2, V_4 and V_5, characterized by the association of a cytokinine (BAP or kinetine) with GA_3, or only by the presence of a cytoquinine (zeatine). The association of cytokinine with an auxine (NAA, IAA), the report being 2 to 1, brought a rhizogenic evolution of the formed meristematic centers.

In order to develop the root system there is no need to introduce phytohormones in the culture medium. The NAA presence in a concentration of 0,5 mg/l determines the development of an abnormal radicular system, with very thick and knotty tending to form callus.

The embryo structures were formed only in callus. There wasn't observed on any variant of medium or on any genotype of those tested the appearance of andro- and gynogenetic formations right from anthers or ovary.

The cytogenetic study done on 15 gametoclones showed a high frequency of haploids (67%) compared with diploid regenerated plants (33%) and the absence of other ploidy degrees.

The encouraging results obtained make us hope that further researches in this direction would solve to tomatoes the problem of obtaining haploids and dihaploids by unconventional means, as they are useful for the amelioration of this culture species.

SUMMARY

In order to induce experimental androgenesis and gynogenesis to tomatoes (*Lycopersicon esculentum* Mill.) there were used anthers with pollen in the uninucleated stage and unfertilised ovaries belonging to 15 genotypes.

Out of the 15 genotypes of tomatoes tested for their ability of forming haploids plants "in vitro", two of them (L_6 and L_7) presented both androgenetic and gynogenetic aptitude, the L_3 genotype produced plants from anthers, but L_2, L_5 and $W_1$from ovaries.

An equal proportion of auxine and cytokinine (1:1) favors the induction of callus, but also it's evolution to embryos and plants from anthers. The association of cytokinine with GA_3 or only in the presence of cytokinine allows the callus evolution gynogenetically in embryos and plants. The rapport 2 to 1 between cytokinine and auxine determined a rizogenetic evolution of the formed meristematic centres. In order to develop the radicular system is not necessary to introduce phytohormones in the culture medium.

The preliminary cytogenetic study done on 15 gametoclones showed a high frequency of haploids (67%) compared with the regenerated diploid plants (33%) and the absence of other ploidy degrees.

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