SCIENTIFIC CONTRIBUTIONS

CYTOLOGICAL AND HYSTOLOGICAL CHARACTERIZATION OF SUNFLOWER HYPOCOTYL EXPLANTS

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INTRODUCTION

It has been previously suggested that segregational events (somatic meiosis and prophase reduction) in carrot somatic cells cultured in vitro could be related to the commitment to somatic embriogenesis (Nuti Ronchi,1990;Nuti Ronchi et al.,1990). To test the diffusion of these cytological phenomena among different plant species,hypocotyl explants of Helianthus annuus L. were cultured in liquid B5 medium in the presence of 2,4 D following the same culture protocol of carrot.

MATERIALS AND METHODS

Seeds of sunflower (Helianthus annuus L.) cv Gloriasol were sterilized for 30 min. in 50% NaOCl, then after peeling in 5% NaOcl and rinsed in sterile distilled water.Seeds were then germinated on agarised Gamborg's (1968) B5 medium in darkness at 24±1°C.After 7 days the hypocotyls were dissected as follow:a)the whole hypocotyl was cut in small pieces (2-3 mm) b) the hypocotyl was dissected in three segments corresponding to the upper (cotyledon) medium and lower (root) part of it.Then sections were cultured (0.5 g. hypocotyl/ 25 ml medium) in liquid B5 medium additioned with 2,2 µM 2,4 D (Dichlorophenossyacetic acid).All the cultured were kept in flasks at 24°C on rotary shaker (80 rpm) under continous light (6-12 mol m-2 s-1).Morphological, cytological and hystological analysis were performed on hypocotyl explants fixed at different times of culture in ethanol-acetic acid (3:1 v/v).For hystological analysis hypocotyl samples previously fixed, were dehydrated in alcohol series, cleared in xylol and embedded in paraffin.Ten µm sections were cut on a microtome and stained with Feulgen, Feulgen-Hematoxylin, Safranin-Fast green.

RESULTS

Cytological analysis (tab.1) showed the occurrence of segregational events during the first days of culture.In particular prophase reduction frequency was about 20 % of total mitosis observed in accordance with previous data in carrot.The segregation mechanism operated at different level of reduced chromosome numbers even lower than seventeen confirming that number n=17 is a secondary basic number, the Helianthus annuus being probably a secondary polyploid (Heiser and Smith,1955;Jackson and Murray,1983).
Hystological analysis revealed that segregational events were located in structures differentiated around vascular strands which could be assimilated to primitive reproductive organs.A concentric series of flower primordia resembling the immature inflorescence of "in vivo" flower head could be found at both ends of the cut surface of sunflower hypocotyls. Differences in structure induction were observed when apical, radical and medium part of the hypocotyl were separatly cultured dimostrating the co-operative action of endogenous and exogenous auxin to the formation of these structures.Experiments of in situ hybridisation with flower specific molecular probes are in progress to verify the true nature of these structures.

CONCLUSIONS

The implication of these findings are that a determinate program leading to floral induction and including a gametophytic phase is initiated any time the system is challenged by an altered environment such a determinate program being expressed, in a hypocotyl portion, by the sole action of growth factors and wounding.Totipotency would be therefore acquired by means of the re-establishment of a gametic conditions.

Time of culture	Total mitosis n°	Total prophases %(*)	Prophases %	Reductional prophases %	Total metaphases %	Metaphases with second.pairing %	C metaphases %	Anaphases %
t2	66	67	52	15	21	12	-	12
t7	102	43	37	6	49	33	8	8
t11	74	62	38	24	35	16	5	3
t15	102	45	31	14	37	16	4	18
t31	96	60	52	8	27	19	2	13

Table 1 Mitotic cycle analysis of sunflower hypocotyls at different times of culture

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