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枇杷属植物野生种的茎段愈伤组织诱导植株再生研究

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摘 要:【目的】建立野生种枇杷茎段愈伤组织诱导植株再生的离体培养技术体系。【方法】以枇杷属植物的2个野生种、1个属间杂交后代为试材,采用茎段为外植体,在不同植物生长调节剂的组合配比培养条件下,开展野生枇杷茎段诱导愈伤组织以及愈伤组织诱导出不定芽的植株再生研究。【结果】最适合野生种枇杷茎段愈伤组织诱导植株再生的培养基配方为:MS+6-BA 1.0 mg·L^{-1}+TDZ 0.1 mg·L^{-1}+NAA 0.1 mg·L^{-1},如栎叶枇杷茎段愈伤组织诱导植株再生,其愈伤率、丛芽率和成苗率均达到100%,不定芽数最多可达38.75个;但对于属间杂交后代的石斑木×台湾枇杷最适合茎段愈伤组织诱导植株再生的培养基配方为:MS+6-BA 1.0 mg·L^{-1}+NAA 0.1 mg·L^{-1},其丛芽率和芽苗数量分别为100%和48个,成苗率也为100%;在众多的生长调节剂中,TDZ对野生枇杷的愈伤组织诱导再分化或脱分化是比较敏感的,高浓度的TDZ虽有利于愈伤组织诱导再分化不定芽,但不利于成苗。【结论】研究得出枇杷属植物以及属间杂交后代茎段愈伤诱导植株再生的共性规律和种间的差异性,建立起枇杷属植物野生种离体再生系统,简化植株再生诱导的步骤和缩短植株再生诱导的时间,能在短时间内产生一定数量的组培苗,为枇杷属种质资源研究积累了新的资料。

关键词:枇杷属植物;野生种;茎段;离体培养;愈伤组织;植株再生

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Study on plant regeneration of callus from the stem of wild loquat

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Abstract:【Objective】The *Eriobotrya* Lindl. belongs to the genus Maloideae of the Rosaceae subfamily. The loquat originates in the south of China and some countries in Southeast Asia, which are adjacent to the southwest of China and there are more than 30 species or varieties. Breeding of wild loquat and quantitative cultivation test work has just begun. The lack of a system of rapid propagation system, especially the explant material used in tissue culture of wild loquat, is scarce. The stem, which is relative to the wild loquat whole plant, is at the most convenient and rich place. Therefore it is necessary to establish the *in vitro* culture system for the regeneration of the stem callus of wild loquat. The study on wild loquat stem covers callus induction, bud induction and plantlet formation of adventitious bud growth and regeneration, in order to find suitable methods for induction of stem segment buds, adventitious bud and seedling culture medium combination formula and environmental growth conditions.【Methods】This work used the stem of 2 wild species of *Eriobotrya* Lindl., and 1 interspecific hybrid as materials to study the callus induction and plant regeneration of wild loquats in the different combinations of plant growth regulators and culture conditions. The two wild species are *E. prinoides* Redh. & Wils and *E. bengalensis* f. *angustifolia* Vidal, which were taken from the Yunnan province of China's Shiping county and Kunming city. In addition, a

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hybrid between the two species of the genus *Rhaphiolepis indica*×*E. deflexa* Nakai is a new species of Loquat Germplasm Resources in the South China Agricultural University.【Results】The optimum medium for inducing plant regeneration from callus of the stem segment of the wild species of *Eriobotrya* plants is MS+ 6-BA 1.0 mg·L^{-1} + KT 1.0 mg·L^{-1} + NAA 0.1 mg·L^{-1}. For example, in the regeneration of callus from the stems of *E. prinoides* Redh. &Wils, the induction rate, sprouting rate and plantlet rate are all 100%, the maximum number of adventitious buds is 38.75. The *E. bengalensis* f. *angustifolia* Vidal of callus and Plantlet rate reached 100%, sprouting rate reached 65.91% and the number of adventitious buds was 2.32. However, for the intergeneric hybrid of *Photinia serrulata* Lindl.×*E. deflexa* Nakai, the best medium component for the generation of the stem callus are MS+6-BA 1.0 mg·L^{-1} + NAA 0.1 mg·L^{-1} and MS+ 6-BA 1.0 mg·L^{-1} + TDZ 0.1 mg·L^{-1}+ NAA 0.1 mg·L^{-1}, the plantlet regeneration of formula MS+ 6-BA 1.0 mg·L^{-1} + TDZ 0.1 mg·L^{-1}+ NAA 0.1 mg·L^{-1} to the stem callus of *E. prinoides* Redh. &Wils and *E. bengalensis* f. *angustifolia* Vidal of the most suitable formula screening results are basically the same, but the MS+ 6-BA 1.0 mg·L^{-1} + NAA 0.1 mg·L^{-1} from the late train performance is better in the formula between the two, where the sprouting rate is 100% and the number of adventitious buds is 48 and the plantlet rate is 100%. Among the many growth regulators, TDZ is more sensitive to callus induction and differentiation of the wild loquat, in the high concentration of TDZ, although the differentiation of adventitious buds to induce callus is also not conducive to sprouting.【Conclusion】In different types of wild loquat experiments, the plant regeneration induced effect on both show some similarities, but also show some differences. This may be related to the relationship between the gene and species of wild loquat, and Liu Yicun (2014, 2016) in *Eriobotrya* species embryo, leaf and stem tip culture *in vitro* in a series of reports of interspecific similarities and differences appear to be similar. The test found that grouper *Photinia serrulata* Lindl. × *E. deflexa* Nakai crossbred loquat *in vitro* seedling and easy propagation coefficient, and in the preliminary test, the *in vitro* culture of grouper *Photinia serrulata* Lindl. is also relatively easy to induce and the seedling propagation coefficient is also large, but the wild species of *E. deflexa* Nakai is not. Thus, *Photinia serrulata* Lindl. may have some genetic improvement effects on the reproduction ability of wild loquat. The wild loquat germplasm resources are low, it is easy to brown, slow breeding, and the tissue culture is difficult; compared with other genera of Rosaceae plant the research is relatively backward. A lot of wild loquat germplasm resources are scarce, so they cannot be studied in depth, such as drug extraction, germplasm conservation, breeding and transgenic research. A relatively reliable *in vitro* regeneration system of the wild species of the genus loquat was established, which simplified the steps of plant regeneration induction and shortened the time of plant regeneration induction, during a short period of time to produce a certain number of tissue culture seedlings, in order to provide the basic material and technical basis for further research on germplasm conservation, breeding and transgenic research.

Key words: *Eriobotrya* plants; Wild species; Stem; *In vitro* culture; Callus; Plant regeneration

枇杷属植物(*Eriobotrya* Lindl.),隶属蔷薇科(Rosaceae)苹果亚科(Maloideae)。原产我国南方地区或与我国西南毗邻的东南亚国家,共有30余个种或变种。其中,普通枇杷(*E. japonica* Lindl.)不仅是亚热带的特色果树,也是重要的传统中药材[1-3]。对于普通枇杷的栽培管理、贮藏加工、组织培养、分子生物学和叶片药用价值等方面研究较多,而栽培枇杷之外的多数枇杷属植物研究较少,并且多年来的调查发现,野生枇杷资源正逐渐减少[4-6]。

关于枇杷属植物离体培养再生植株的研究主要集中在普通枇杷[7]。自普通枇杷茎尖培养获得再生植株成功后[8],胚培养、胚乳培养、原生质体培养以及胚状体诱导等技术相继建立[9-15]。近10年来,枇杷的离体诱导培养不断取得一些新进展,如利用容易