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EFFECT OF NAPHTHENIC ACIDS ON FORMATION OF ADVENTITIOUS ROOTS IN SUNFLOWER CUTTINGS

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SUMMARY

The paper describes a study of the effect of salts of natural naphthenic acids on the rooting of young sunflower cuttings and latteral branching of interspecies sunflower hybrids. Naphthenic acids were obtained by alkaline extraction from atmospheric gas oil fraction of Vojvodina crude oil "Velebit" and purified by column chromatography on alumina. Their sodium salts in concentrations of 1×10^{-7} mol/dm^3 stimulated the formation of adventitious roots in sunflower cuttings even by a factor of 40 compared with control, the effect being also observed in lateral branches of interspecies sunflower hybrids. The obtained results suggest the possibility of using naphthenic acids as a means for rooting of plant cuttings.

Key words: naphthenic acids, adventitious root, sunflower

INTRODUCTION

Napthenic acids represent a complex mixture of acids obtained from oil and oil derivatives by alkaline extraction.

In addition to a wide application in the chemical industry, these compounds exhibit biological activity which has been known since the middle of the previous century (Lochte and Littmann, 1955). Thus it has been noticed that mixtures of natural naphthenic acids can influence the yield and increase dry mass of plants (Kuliev *et al.,* 1965; Fattah and Wort, 1970; Severson, 1971), nitrogen metabolism (Wort *et al.,* 1973), photosynthesis and respiration (Kastori *et al.,* 1988).

It is known that naphthenic acid can also act as a regulators of plant growth (Parups, 1969), which has been confirmed in our previous works, reporting that

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mixtures of natural naphthenic acids isolated from middle and higher fractions of the Vojvodina crude oil "Velebit" exhibited physiological activity analogous to that of plant hormones of auxin and gibberellin type (Ćirin-Novta *et al.,* 2002; Vasić *et al.*, 2002).

With most of plants, rooting occurs in the presence of plant hormones. Thus, soon after the discovery of indol-3-acetic acid, a natural auxin, it was found that it stimulates formation of adventitious roots in plants (Went *et al.*, 1937). Synthetic auxins such as indol-3-butyric acid and naphthyl-acetic acid exhibit an enhanced efficiency in the induction of adventitious root formation, especially with plants that are hard to root (Audus, 1959). The majority of the commercially available preparations of plant hormones intended for rooting contain indol-3-butyric or naphthyl-acetic acid, or mixtures of these substances with indol-3-acetic acid.

In view of our previous findings that the mixtures of natural naphthenic acids isolated from middle and higher oil fractions of Vojvodina crude oil "Velebit" exhibited physiological activity analogous to that of plant hormones of auxin type, and having in mind the known role of auxin in root formation, it was hypothesized that naphthenic acids should also influence the process of root formation in sunflower cuttings.

The aim of this work was to investigate the effect of salts of natural naphthenic acids on root formation in cuttings of young sunflower plants and rooting of lateral branches of interspecies sunflower hybrids.

MATERIAL AND METHODS

Isolation and characterization of naphthenic acids

Isolation of naphthenic acids from oil fractions of Vojvodina crude oil "Velebit", their characterization by IR spectroscopy and GC-MS analysis, as well as preparation of their sodium salts were described in our previous papers (Ćirin-Novta *et al.,* 1994; Ćirin-Novta *et al.,* 2002).

Rooting of sunflower cuttings

Sunflower plants were obtained by germinating the seeds of the hybrid NS-H-1304 in sand, where seedlings were grown to a height of about 15 cm, i.e., to the formation of first pair of leaves. Plant roots were removed by cutting them 1 cm above the root neck. Batches of 10 plants were then immersed to a hieght of about 6 cm in water (control) and in the solutions of different concentrations (1×10^{-3}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7} and 1×10^{-8} mol/dm^3) of sodium salts of naphthenic acids. After 12 days of growing in the greenhouse (temperature 25°C; photoperiod 16 hours; air humidity 80%; occasional water added to compensate for loss by evaporation), the newly formed adventitious roots (all longer than 2 mm) were counted.

The obtained results are presented as a mean number of adventitious roots per plant.

The presented data are mean values of three repetitions and they were subjected to the analysis of variance by the method of lowest significant difference.

Rooting of sunflower lateral branches

Experiments were carried out using lateral branches of sunflower plants obtained by interspecies crossing over within the genus *Helianthus*. Lateral branches of the plants were cut at the stage of appearance of the first pair of leaves, i.e., when they were about 3 cm long. After cutting, the branches were planted into sand and treated with distilled water (control) and with solutions of preparations of sodium salts of naphthenic acids in the concentration of 1×10^{-7} mol/dm^3. Plants were grown under controlled conditions (25°C and photoperiod 16 hours). After two weeks, the plants were taken out from the sand and examined for the presence/absence of roots, i.e., to establish the percentage of the plants having adventitious roots.

RESULTS AND DISCUSSION

Partial immersion of sunflower cuttings into solutions of sodium naphthenates stimulated the formation of adventitious roots and the numbers of roots per plant were as much as 40 times higher compared with control plants (Figure 1).

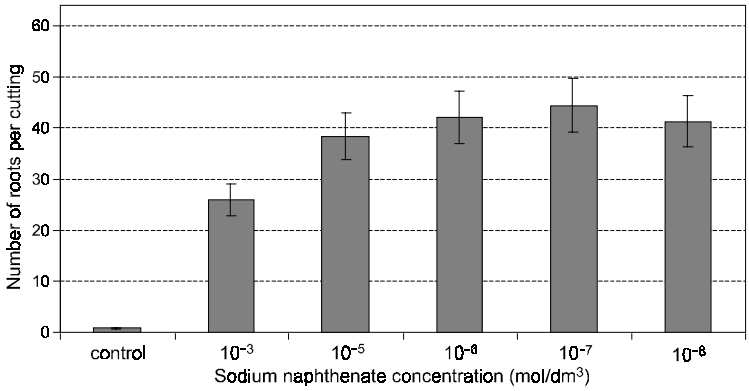

Figure 1: Effect of sodium naphthenate concentration on formation of adventitious roots in sunflower cuttings. Bars represent standard error

The largest number of adventitious roots per plant was formed at lower concentrations of sodium naphthenate, i.e., from 1×10^{-5} to 1×10^{-8} mol/dm^3, the differences in the number of roots per plant in this concentration range being non-significant. At the highest concentration of sodium naphthenate (1×10^{-3} mol/dm^3), the numbers of adventitious roots formed was significantly lower compared with those formed at lower concentrations, on the average by 60%.

A comparison of our results with those obtained by Fabijan *et al*. (1981), who found that treatment of sunflower hypocotyls with indol-acetic acid in the concentration of 1×10^{-6} mol/dm^3 stimulated initiation of adventitious roots while higher concentrations (1×10^{-4} mol/dm^3) showed an inhibitory effect, provides ground for the conclusion that sodium naphthenate acts similar to auxin. In their experiments with sunflower hypocotyls Liu and Reid (1992) concluded that auxin is the primary agent which controls formation of adventitious roots.

Results quite similar to the above were also obtained in the second group of experiments, involving sunflower lateral branches replanted in sand and treated with solution of sodium naphthenate in a concentration of 1×10^{-7} mol/dm^3. In this investigation, sodium naphthenate exhibited also a highly favorable effect on root formation (Figure 2). In addition to root formation, this treatment had also an indirect positive effect on the growth and development of the plants, i.e., increase of dry matter mass.

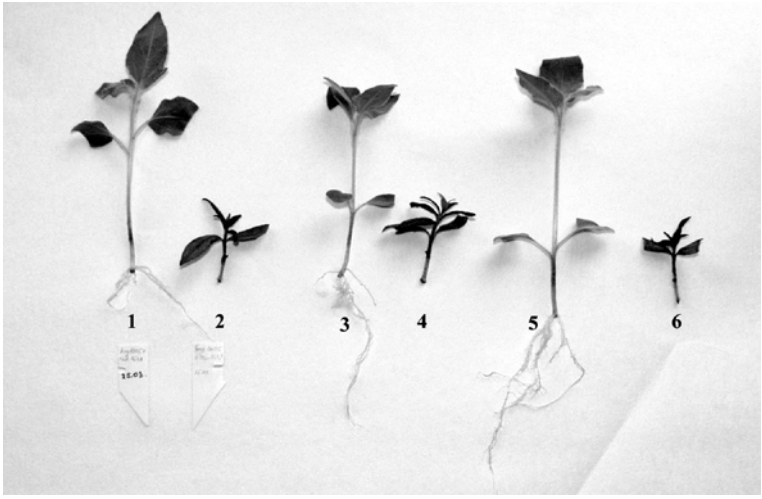

Figure 2: Effect of sodium naphthenate on root formation with three different sunflower genotypes. Plants 1, 3 and 5 were treated with sodium naphthenate solution and plants 2, 4 and 6 are plants of the same genotype treated with water (controls)

Treatment with sodium naphthenate showed high efficiency as can be judged from the proportion of rooted plants: with nine sunflower genotypes it was in the range from 40 to 100% (78.5% on the average) (Table 1). The effect of sodium naphthenate solution on root formation from sunflower lateral branches was different with particular sunflower genotypes, which could be explained in terms of genetic differences. It is possible that the investigated genotypes differ in the content of the light-induced substance, found in sunflower seedlings, which inhibits the auxin action (Yokotani-Tomita *et al*., 1997). This is another proof of the hypothesis that sodium naphthenate action is similar to that of auxin. Besides, some authors reported that it is necessary to ensure the appropriate concentration, and even the

type of both natural and synthetic auxins for particular plant genotype to achieve its effective rooting (Vasić *et al.*, 2001; Dhaka and Kothari, 2002).

Table 1: Efficiency of rooting of lateral branches of different sunflower genotypes treated with a 1×10^{-7} mol/dm^3 solution of sodium naphthenate

Genotype	Proportion of rooted lateral branches (%)
Ha-48 × tub7	100
arg1805 × max1631	100
gig1605 × max1631	100
Ha-26A × dec2053	40
PHBC1-212 × deb1810	50
Ha-26A × pet2122	100
PHBC1-193A × pet1910	50
max1631 × arg1805	67
Ha-26A × pet1383	100

In addition to natural and synthetic plant growth regulators, some other factors too can stimulate the development of adventitious roots, for example, lower pH value of the substrate (Liu *et al.,* 1993), boron (Josten and Kutschera, 1999), 2-chloroethyltrimethylammonium chloride (Misra and Pradham, 1968) and piperazine (Liu *et al.*, 1995). In this work we showed for the first time that the mixture of natural naphthenic acids isolated from the atmospheric gas oil fraction of Vojvodina crude oil "Velebit" can have a favorable effect on root formation in plant cuttings. Most probably, the mixture of naphthenic acids obtained from these fraction (which is characterized by a mass ratio of C atoms in cyclic structures of 62.1%, in aliphatic structures 33.7% and in aromatic structures of 4.4%, with an average number of C atoms in a molecule being 15 and average molar mass of 243) has a favorable composition that stimulates root formation. The obtained results are of great practical importance as naphthenic acids could be used for this purpose, which has been the subject of a pending patent (Kevrešan *et al.*, 2002).

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INFLUENCIA DE LOS ÁCIDOS NAFTÉNICOS EN FORMACIÓN DE RAÍCES ADVENTICIAS EN EL ESQUEJE DE GIRASOL

RESUMEN

En el trabajo se investigaba la influencia de la sal de los ácidos nafténicos naturales en el arraigamiento de los esquejes de girasol jóvenes y de ramas laterales de interespecies de híbridos de girasol. Los ácidos nafténicos se obtuvieron por la extracción alcalina de la fracción gaseosa-oleosa atmosférica del petróleo crudo de Voivodina, Velebit y purificada por cromatografía en la colona de óxido de aluminio. Sus sales de sodio en concentración de 1×10^{-7} mol/dm^3 estimularon la formación de raíces adventicias en los esquejes de

girasol, que incrementó hasta 40 veces en relación con el control. Tal influencia se notó también en las ramas laterales de interespecies de híbrido de girasol. Los resultados obtenidos indican la posibilidad de utilización de los ácidos nafténicos como medio de arraigamiento de los esquejes de las plantas.

EFFET DES ACIDES NAPHTÉNIQUES SUR LA FORMATION DE RACINES ADVENTIVES DANS LES BOUTURES DE TOURNESOL

RÉSUMÉ

Cet article décrit une étude sur l'effet des sels des acides naphténiques naturels sur la formation de racines des jeunes boutures de tournesol et sur les branches latérales des hybrides interspecies de tournesol. Les acides naphténiques ont été obtenus par extraction alcaline du fractionnement atmosphérique de gaz et d'huile du pétrole brut de Vojvodine Velebit et purifié par chromatographie sur une colonne d'oxyde d'aluminium. Les sels de sodium en concentration de 1×10^{-7} mol/dm^3 ont stimulé la formation de racines adventives dans les boutures de tournesol qui ont augmenté jusqu'à 40 fois par rapport au contrôle. Un effet semblable a aussi été observé dans les branches latérales des hybrides interspecies de tournesol. Les résultats obtenus indiquent la possibilité d'utiliser les acides naphténiques comme moyen d'enracinement de boutures de plantes.