Effects of different cytokinins on the shoot regeneration from apple leaves of 'Royal Gala' and 'M.26'

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Summary: The effects of different types of cytokinins on the shoot regeneration from leaf explants of apple scion 'Royal Gala' and apple rootstock 'M.26' were evaluated. Regeneration media contained either thidiazuron, or 6-benzylaminopurine, or meta-topolin, or zeatin, or kinetin, or their N^9-ribosides, respectively, in the concentration range 0.5 to 8.0 mg l^{-1}. Effects of these cytokinins were evaluated on the percentage of regeneration (R%) and that of vitrification (V%) and on the number of regenerated shoots per explant (SN). Organogenetic index (OI) calculated from these data was used for the evaluation of efficacy of cytokinins. The course of shoot organogenesis also was followed using stereomicroscope. Types and concentrations of cytokinins applied in the regeneration media influenced each parameter significantly and the regeneration answer was strongly genotype-dependent. The best regeneration (SN: 11.08, OI: 7.5) was achieved in 'Royal Gala' by using TDZ in concentration of 0.5 mg l^{-1} (2.27 µM). There was a clear relationship between the effect on the regeneration efficacy and the chemical structure of cytokinins considering classical cytokinins, namely N^9-ribosides applied in less concentration than non-ribosides have the same or best regeneration effects except for 6-benzylaminopurine riboside. However, similar relationship could not be detected in the case of 'M.26'. SN was the highest (3.22) using 6.5 mg l^{-1} (18.20 µM) 6-benzylaminopurine riboside or 8.0 mg l^{-1} (21.44 µM) meta-topolin riboside (3.18). SN was not significantly lower (3.12) by using 2.0 mg l^{-1} (9.08 iM) TDZ, however, OI was about half as big (0.63 compared to 1.29 or 1.74 with 6-benzylaminopurine riboside or meta-topolin riboside, respectively). 'Royal Gala' had higher organogenetic ability, than 'M.26': 3.5-fold higher shoot number per explant and more than 4-fold higher organogenetic index was reached with this cultivar than with 'M.26'. Moreover, the similar developmental stage of shoots could be observed 3–5 days earlier than in 'M.26' and if explants of 'Royal Gala' were further cultured with 3 weeks, SN increased from 11.08 to 24.42 on TDZ-containing regeneration medium, which might suggest higher organogenetic ability, too.

Abbreviations: BA = 6-benzylaminopurine, BAR - 6-benzylaminopurine riboside, KIN = 6-furfurylaminopurine or kinetin, KINR = kinetin riboside, NAA = 1-naphtil-acetic-acid, TDZ = N-fenil-N'-1,2,3-thidiazol-5-il-urea or thidiazuron, TOP = 6-(3-hydroxybenzylamino)purine or meta-topolin, TOPR = meta-topolin riboside, ZEA = N^6-((E)-4-hydroxy-3-methylbut-2-enyl)adenine or zeatin, ZEAR = zeatin .

Key words: apple, cytokinins, shoot regeneration, organogenetic ability

Introduction

Effective regeneration from tissues of *in vitro* fruit plants is a necessary precondition for the implementation of different biotechnological approaches in plant breeding. (*James & Dandekar,* 1991, Korban et al., 1992). Numerous studies have been reported about regeneration of apple and organogenesis has proved to be influenced by several factors, such as age and source of explant, hormonal balance of the regeneration medium, culture conditions, type of vessel, etc. (*Dufour,* 1990, *Famiani* et al., 1994, *Fasolo* et al., 1989, *Ferradini* et al., 1996, *James & Dandekar,* 1991, *James* et al., 1988, *Korban* et al., 1992, *Predieri & Fasolo,* 1989, *Sriskandarajah & Goodwin,* 1998, *Standardi & Houshmand,* 1992, *Theiler-Hedtrich & Theiler-Hedtrich* 1990, *Welander,* 1988, *Yepes & Aldwinckle,* 1994).

However, one of the most important factors during regeneration process is the type and concentration of cytokinin applied. Cytokinins are N^6-substituated adenines with growth regulatory activity in plants. There are two main classes of them: isoprenoid and aromatic cytokinins, which differ from each other in their biochemistry, their receptors, their biological activity and their metabolism (*Strnad* et al., 1997, *Werbrouck* et al., 1996).

In general, good regeneration could be obtained in several apple genotypes using BA or TDZ as cytokinin (*Fasolo* et al., 1989, *Korban* et al., 1992, *Sriskandarajah* et al., 1990, *Swartz* et al., 1990, *Yao* et al., 1995). However, several undesirable side effects of TDZ, such as vitrification, forming of fasciated shoots, etc., were obtained mainly in higher than 1 µM (0.2 mg l^{-1}) concentration (*Caboni* et al., 1996, *Huetteman & Preece,* 1993, *Pawlicki & Welander,* 1994), therefore its use should be avoided where classical, natural cytokinins work.

The aim of present work was to evaluate the effects of different types of cytokinins in the induction of shoot regeneration from leaf explants of apple scion 'Royal Gala' and apple rootstock 'M.26'. We considered the effects of

different aromatic cytokinins, namely: BA, which has benzylamino side chain; KIN with furfurylamino side chain; TOP, which is a hydroxylated analogue of BA; ZEA, which is one of the most active isoprenoid cytokinins; furthermore their N^9-ribosides (BAR, TOPR, ZEAR, KINR).

Material and method

Regeneration experiments

After three-week-long pre-treatment on media containing 1.0 mg l^{-1} TOP (*Dobránszki* et al., 2002), the upper two, fully-expanded young leaves were used for regeneration. Petiole and apex of leaves were removed and leaves were cut transversely into two stripes (about 5 mm wide). All cutting were made in a solution of citric acid (0.15 g l^{-1}) and ascorbic acid (0.1 g l^{-1}). Leaf explants were placed then with the adaxial side onto the regeneration media. Regeneration media consisting of MS salts (Murashige & Skoog, 1962), B_5 vitamins supplemented with 100 mg l^{-1} myo-inositol, 0.25% gelrite, 3% sucrose, 0.2 mg l^{-1} NAA and the different types of cytokinins (TDZ, BA, KIN, TOP, ZEA, BAR KINR, TOPR and ZEAR), respectively, in the concentration range 0.5 to 8.0 mg l^{-1} *(Table 1)*. Explants were incubated in the dark at 24.5 °C for 3 weeks, then in the light at 22 °C with 16 h photoperiod. The light intensity was increased weekly: it was 35 µmol s^{-1} m^{-2} during the first week, 70 µmol s^{-1} m^{-2} during the second week and 105 µmol s^{-1} m^{-2} from the third week.

After seven and after ten weeks the number of explants with regenerated shoot (=regeneration percent, R%), the number of regenerated shoots per explant (SN), and the number of explants with vitrified shoots (=vitrification percent, V%) were recorded. The data were analysed statistically by ANOVA followed by Tukey's test by using *SPSS 7.5 for Windows* software. The data given in percent (R%, V%) were non-parametric, therefore they were analysed after arcsine transformation. The results are presented in non-transformed format. Experiments were repeated twice in time. From the data observed 'Organogenetic Index' (OI) was calculated, as follows: OI = (R% – V%) x SN / 100.

Time change of shoot organogenesis during culturing

The course of shoot organogenesis was followed using stereomicroscope from the first day of culturing explants in the light, which means the 22nd day after the beginning of stimulation with one kind of cytokinins, till detecting clear differences in the developmental stage of organogenesis between the different regeneration media and/or between the cultivars. The first day of observations was determined in pre-experiments. For these examinations we chose the optimal concentration of TDZ, the optimal concentration of two classical cytokinins with the best effects on SN and OI and the optimal concentration of classical cytokinin with the lowest effects on SN and OI in both cultivars. Accordingly, early stage of organogenesis was examined on regeneration media contained 0.5 mg l^{-1} TDZ, 5.0 mg l^{-1} BA, 6.5 mg l^{-1} TOPR and 6.5 mg l^{-1} KIN in the case of 'Royal Gala' and 2.0 mg l^{-1} TDZ, 6.5 mg l^{-1} BAR, 8.0 mg l^{-1} TOPR and 5.0 mg l^{-1} KIN in the case of 'M.26', respectively.

Table 1. Types and concentrations of cytokinins applied in the regeneration media

Type of cytokinin	Applied concentrations of cytokinins mg l–1 (µM)					
TDZ	0.5 (2.27)	2.0 (9.08)	3.5 (15.89)	5.0 (22.70)	6.5 (29.51)	–
BA	0.5 (2.22)	2.0 (8.88)	3.5 (15.54)	5.0 (22.20)	6.5 (28.86)	–
BAR	0.5 (1.40)	2.0 (5.60)	3.5 (9.80)	5.0 (14.00)	6.5 (18.20)	8.0 (22.40)
TOP	0.5 (2.07)	2.0 (8.28)	3.5 (14.49)	5.0 (20.70)	6.5 (26.91)	–
TOPR	0.5 (1.34)	2.0 (5.36)	3.5 (9.38)	5.0 (13.40)	6.5 (17.42)	8.0 (21.44)
ZEA	0.5 (2.28)	2.0 (9.12)	3.5 (15.96)	5.0 (22.80)	6.5 (29.64)	–
ZEAR	0.5 (1.42)	2.0 (5.68)	3.5 (9.94)	5.0 (14.20)	6.5 (18.46)	8.0 (22.82)
KIN	0.5 (2.32)	2.0 (9.28)	3.5 (16.24)	5.0 (23.20)	6.5 (30.16)	–
KINR	0.5 (1.44)	2.0 (5.76)	3.5 (10.08)	5.0 (14.40)	6.5 (18.72)	8.0 (23.04)

Table 2. Effects of TDZ on the shoot regeneration of 'Royal Gala' and 'M.26' after 7-week-long regeneration*

Applied concent-rations mg l^{-1} (µM)	'Royal Gala'				'M.26'			
	SN	R%	V%	OI	SN	R%	V%	OI
0.5 (2.27)	11.08 c	98.3 ab	34.7 a	7.05	2.74 a	76.2 b	59.8 b	0.45
2.0 (9.08)	5.93 b	100.0 b	88.2 b	0.70	3.12 a	54.8 ab	34.7 ab	0.63
3.5 (15.89)	6.12 b	100.0 b	86.6 b	0.82	2.22 a	44.7 a	36.5 ab	0.18
5.0 (22.70)	5.20 b	93.2 ab	58.2 ab	1.82	2.61 a	54.6 ab	23.0 a	0.82
6.5 (29.51)	3.12 a	84.9 a	89.9 b	0	2,58 a	39,8 a	19.8 a	0.52

*The statistically homogeneous groups are indicated in every type of cytokinins in each apple cultivar by the same letter.

Results

Regeneration experiments

Results after 7-week-long regeneration are presented in *Tables 2–4*. Each parameter measured were significantly influenced by the types and concentrations of cytokinins applied in the regeneration media. SN did not increase when explants were further-cultured for 3 weeks, except in the case of Royal Gala, if TDZ was used in the regeneration media. As a result of further culturing, SN doubled (not presented in the tables).

In the case of 'Royal Gala' the R% was the highest (90–100%) if TDZ, BA or BAR was used. TOP, TOPR, ZEA or ZEAR induced regeneration in 63–81% but KIN or KINR caused very low R% (under 45%). Very high (34.7–89.9%) vitrification was detected if TDZ was used for regeneration. When TOP, KIN or KINR was used for inducing shoot regeneration, no any vitrification was detected similarly to the finding of others (*Dobránszki* et al, 2002, *Kubalákova & Strnad,* 1992). In the case of other cytokinins tested, the V% was very low or manifested only after higher (>3.5 mg l^{-1}) concentrations of cytokinins.

The number of regenerated shoots per leaf explant (SN) was the highest (11.08) after 0.5 mg l^{-1} (2.27 μM) TDZ treatment, however one third of the shoots showed vitrified characters (*Fasolo* et al., 1989, *Huetteman & Preece,* 1993). The next highest SN was achieved by use of 5.0 mg l^{-1} (22.2 μM) BA (SN: 4.58) or 6.5 mg l^{-1} (17.42 μM) TOPR (SN: 3.48). The least shoots developed on media contained KIN or KINR. *Figure 1* presents the SN and OI at the optimal concentration of different cytokinin types in the case of 'Royal Gala'.

In the case of 'M.26' the R% was the highest (above 70%) if TDZ, BA, BAR or TOP was used and it was the lowest (18.5 and 33.2%) after application of KIN or KINR. Vitrification was the highest using TDZ. However, no any vitrification was detected using KIN or KINR or it was very

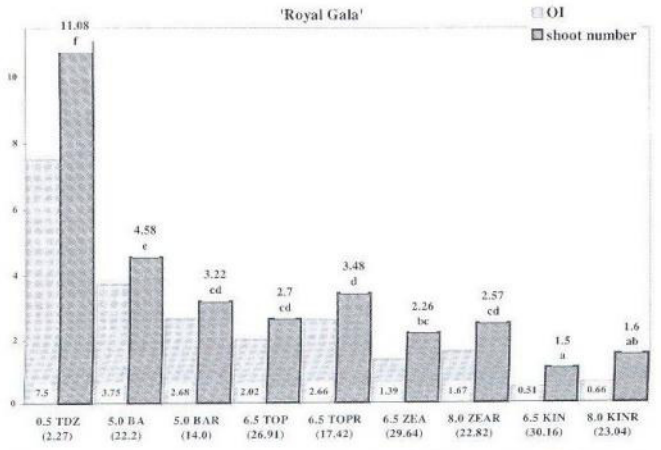

Figure 1. Effects of optimal concentration of different cytokinins on the number of regenerated shoots per explant (SN) and on the organogenetic index (OI) of 'Royal Gala'. (The statistically homogeneous groups are indicated by the same letter.)

Table 3. Effects of aromatic and isoprenoid cytokinins on the shoot regeneration of 'Royal Gala' and 'M.26' after 7-week-long regeneration*

Applied concent-rations mg l^{-1} (μM)	'Royal Gala'				'M.26'			
	SN	R%	V%	OI	SN	R%	V%	OI
0.5 (2.22)	1.22 a	29.70 a	0 a	0.36	1.36 a	36.40 a	0 a	0.50
2.0 (8.88)	3.22 b	84.80 b	0 a	2.73	1.73 ab	73.00 c	36.50 b	0.63
3.5 (15.54)	3.84 bc	94.90 b	0 a	3.64	2.45 b	69.50 bc	14.70 ab	1.34
5.0 (22.20)	4.58 c	94.90 b	13.10 b	3.75	2.00 ab	46.40 ab	1.60 a	0.90
6.5 (28.86)	3.82 bc	96.60 b	14.60 b	3.13	1.32 a	31.20 a	0 a	0.41
KIN								
0.5 (2.32)	0 a	0 a	0	0	0	1.70 a	0	0
2.0 (9.28)	1.00 a	6.50 a	0	0.07	0	1.70 a	0	0
3.5 (16.24)	1.43 a	11.40 a	0	0.16	1.09 a	18.50 b	0	1.09
5.0 (23.20)	1.50 a	16.40 a	0	0.25	1.89 b	15.20 ab	0	1.89
6.5 (30.16)	1.15 a	44.70 b	0	0.51	1.33 a	16.70 ab	0	1.33
TOP								
0.5 (2.07)	1.00 a	6.60 a	0 a	0.07	0	1.60 a	0 a	0
2.0 (8.28)	1.52 ab	34.70 b	0 a	0.53	1.20 a	24.80 b	4.90 a	0.24
3.5 (14.49)	2.17 ab	58.10 c	0 a	1.26	2.26 b	72.90 d	6.40 a	1.50
5.0 (20.70)	2.28 ab	78.00 c	0 a	1.74	2.11 b	64.70 cd	3.20 a	1.30
6.5 (26.91)	2.70 b	74.70 c	4.80 a	2.02	1.63 ab	49.80 c	1.60 a	0.79
ZEA								
0.5 (2.28)	0	0 a	0 a	0	1.00 a	3.20 a	0 a	0.03
2.0 (9.12)	1.33 a	9.80 a	0 a	0.13	1.45 a	18.00 ab	3.20 a	0.21
3.5 (15.96)	1.26 a	37.90 b	0 a	0.48	1.13 a	13.20 ab	0 a	0.15
5.0 (22.80)	1.90 a	51.30 b	1.60 a	0.91	1.25 a	46.40 c	16.50 a	0.37
6.5 (29.64)	2.26 a	63.10 b	3.20 a	1.39	1.50 a	28.00 bc	16.50 a	0.17

*The statistically homogeneous groups are indicated in every type of cytokinins in each apple cultivar by the same letter.

low (under 8%) if TOP or TOPR was used, similarly to reactions of 'Royal Gala' *(Tables 2–4)*.

The number of regenerated shoots per leaf explant (SN) was the highest (3.22) after 6.5 mg l^{-1} (18.20 μM) BAR or after 8.0 mg l^{-1} (21.44 μM) TOPR (3.18) treatments. SN was not significantly lower (3.12) by using 2.0 mg l^{-1} (9.08 μM) TDZ, however, OI was about half as big (0.63 compared to 1.29 or 1.74). Figure 2 presents the R%, V%, SN and OI at the optimal concentration of different cytokinin types in the case of 'M.26'.

Time change of shoot organogenesis during culturing

Leaf explants on regeneration media were cultured for 3 weeks in dark then placed in the light conditions. *Figure 3–4* present the different phases of organogenesis after 1, 3, 7 or 3, 7, 12 days when cultures were placed in the light in 'Royal Gala' and 'M.26', respectively.

In the case of 'Royal Gala' developing shoots could be observed already after the first day in the light if TDZ, BA or TOPR were used for regeneration, respectively. On KIN-containing medium only calli or sometimes root development could be seen *(Figure 3. A, D, G, J)*. The appearance of first shoot primordia could be detected from the 3rd day cultured in the light *(Figure 3. K)*. After 1-week-long culture in the light, well-developed shoots could be detected on TDZ-, BA- or TOPR-containing media but there are differences in the size of shoots because they are appreciably taller on TDZ-containing medium. However, the rate of vitrified shoots is greater on TDZ-containing medium compared to BA- or TOPR-containing media *(Figure 3. C,F,I)*. The developmental phase on KIN-containing medium on the 7th day *(Figure 3. L)* is similar to the phase observable on BA- or TOPR-containing media on the 3rd day *(Figure 3. E, H)*.

The similar developmental phases, which are presented on the *Figure 3* in the case of 'Royal Gala', could be seen on the *Figure 4* in the case of 'M.26'. However, the developmental phases are presented from the 3rd day on the

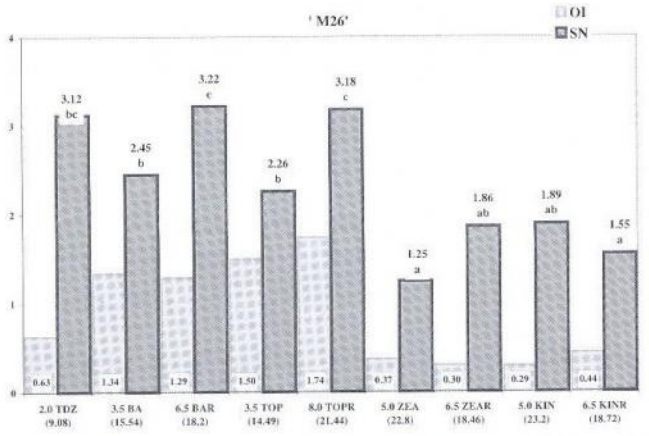

Figure 2. Effects of optimal concentration of different cytokinins on the number of regenerated shoots per explant (SN) and on the organogenetic index (OI) of 'M.26'. (The statistically homogeneous groups are indicated by the same letter.)

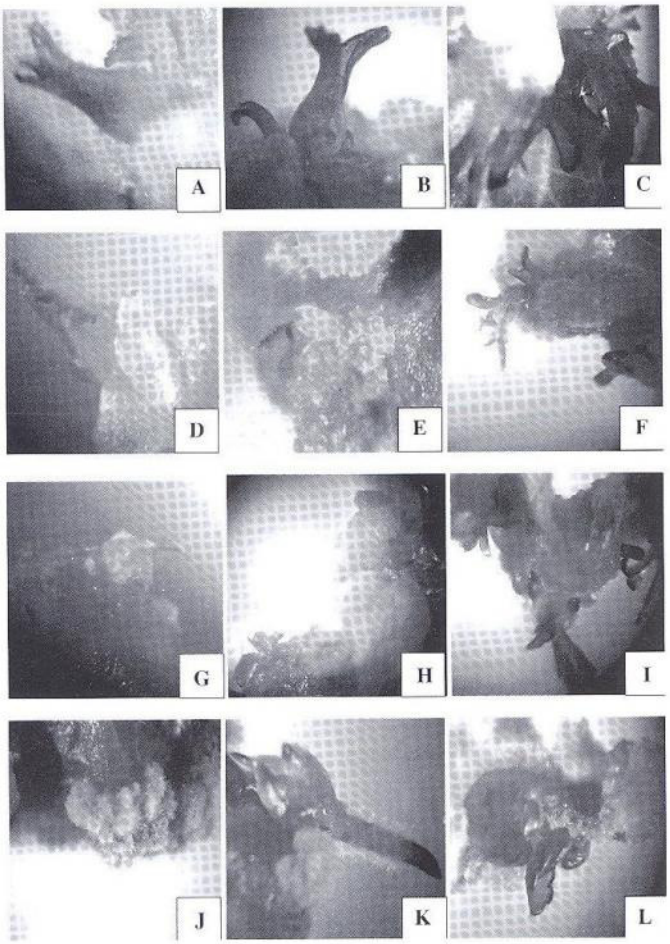

Figure 3. Developmental pattern of shoot regeneration of 'Royal Gala'. After 1 day cultured in the light on media contained 0.5 mg l^{-1} TDZ (**A**), 5.0 mg l^{-1} BA (**D**), 6.5 mg l^{-1} TOPR (**G**), 6.5 mg l^{-1} KIN (**J**) (x 120); after 3 days cultured in the light on media contained 0.5 mg l^{-1} TDZ (**B**), 5.0 mg l^{-1} BA (**E**), 6.5 mg l^{-1} TOPR (**H**), 6.5 mg l^{-1} KIN (**K**) (x 120); after 7 days cultured in the light on media contained 0.5 mg l^{-1} TDZ (**C**), 5.0 mg l^{-1} BA (**F**), 6.5 mg l^{-1} TOPR (**I**), 6.5 mg l^{-1} KIN (**L**) (x 60).

Figure 4, because the morphogenesis of 'M.26' occurred slower than that of 'Royal Gala'. The „backward" of 'M.26' was 3–5 days. Compared the effects of TDZ and BAR or TOPR, development of fasciated and vitrified shoots could be observed on TDZ-containing medium *(Figure 4. A, B, C)*. However, on the BAR- or TOPR-containing media the developed shoots were healthy (non-vitrified) and they were taller *(Figure 4. D-I)* and the shoot development was the slower on KIN-containing medium *(Figure 4. J-L)*, which differences accorded to the differences between the effects of cytokinins could be observed at the end of the regeneration experiments.

Development of new shoots was detected also during the latter phase of culture on regeneration medium contained TDZ in the case of 'Royal Gala' *(Figure 5)*. As a result of this effect of TDZ more than double SN (24.42 instead of 11.08 at 0.5 mg l^{-1} TDZ) could be recorded if explants were cultured with 3 weeks longer on regeneration medium. No any other examined cytokinins caused similar effects on the shoot regeneration of 'Royal Gala'. Moreover, nor TDZ

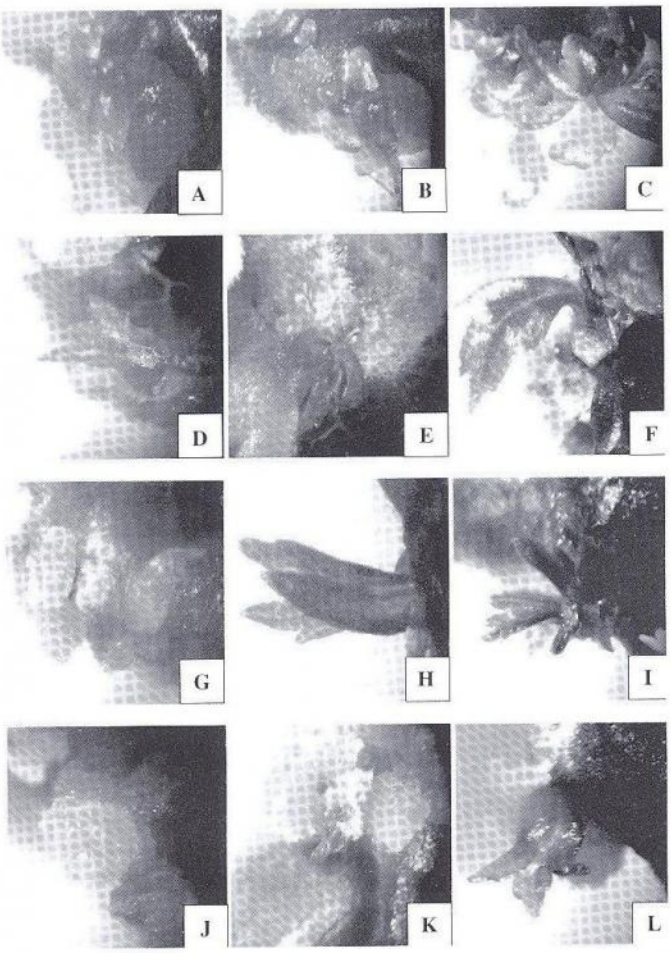

Figure 4. Developmental pattern of shoot regeneration of 'M.26'. After 3 days cultured in the light on media contained 2.0 mg l^{-1} TDZ (**A**), 6.5 mg l^{-1} BAR (**D**), 8.0 mg l^{-1} TOPR (**G**), 5.0 mg l^{-1} KIN (**J**); after 7 days cultured in the light on media contained 2.0 mg l^{-1} TDZ (**B**), 6.5 mg l^{-1} BAR (**E**), 8.0 mg l^{-1} TOPR (**H**), 5.0 mg l^{-1} KIN (**K**); after 12 days cultured in the light on media contained 2.0 mg l^{-1} TDZ (**C**), 6.5 mg l^{-1} BAR (**F**), 8.0 mg l^{-1} TOPR (**I**), 5.0 mg l^{-1} KIN (**L**). (x 120)

caused any increase in the SN in the case of 'M.26' if explants were further cultured (therefore data are not presented here).

Discussion

Our calculation of OI used for evaluation of the effects of different cytokinins in the regeneration process based on the organogenetic index used by *Famiani* et al. (1994) but shoot length was excluded. Moreover, our index includes vitrification percent because of its great importance in the regeneration of normal shoots (*Caboni* et al., 1996, *Dobránszki* et al., 2002, *Huetteman & Preece*, 1993, *Sriskandarajah & Goodwin*, 1998, *Standardi & Houshmand*, 1992).

The highest percent of vitrification was observed when TDZ was applied in the regeneration medium. However, V% was reduced from 88–100% to 34.7% at the lowest TDZ concentration (0.5 mg l^{-1}) by the pre-treatment in 'Royal Gala', as described earlier (*Dobránszki* et al., 2002) and from 72% to 59.8% in 'M.26' (not described earlier). The reaction of the two examined cultivars to the TDZ was significantly different. In the case of 'Royal Gala', the highest number of regenerated shoots and also the highest OI were detected by the use of TDZ. Its effective concentration was 10-times lower than in the case of other cytokinins similarly to the findings of Huetteman & Preece (1993). In our experiments its optimum level was 2.27 μM similarly to the results of *Sarwar & Skirvin* (1997), but contradicting to the results of some other authors (*Fasolo* et al., 1989, *Korban* et al., 1992, Kubalákova & Strnad, 1992), who found the optimal TDZ concentration between 4–10 μM in other apple cultivars *(Tables 2–4 and Figure 1)*. However, the optimal concentration of TDZ (9.08ìM) was found to be in the above mentioned concentration range in the case of 'M.26'. In this rootstock cultivar, the best regeneration results could be reached using BAR or TOPR considering both SN and OI *(Figure 2)*.

Considering the effects of classical cytokinins occurred in the nature, it could be concluded, that the reactions of two tested apple cultivars were different similarly as in the case of TDZ. In 'Royal Gala' the best regeneration (high R%, high SN, low V% and therefore high OI) was achieved if BA, or TOPR was used. N^9-ribosides applied in less concentration than non-ribosides have the same or best regeneration effect (TOP-TOPR, ZEA-ZEAR, KIN-KINR) except for BAR. The best effects were achieved between 22–30 μM in the case of non-ribosides, but between 17–23 μM in the case of N^9-riboside-forms depending of the type of cytokinins *(Figure 1, Tables 3–4)*. Similar relationship between the effect and chemical structure (riboside or non-riboside form) of cytokinins could not be detected in the case of 'M.26' *(Figure 2)*. However, the best regeneration answers were observed if ribosides (BAR or TOPR) were used.

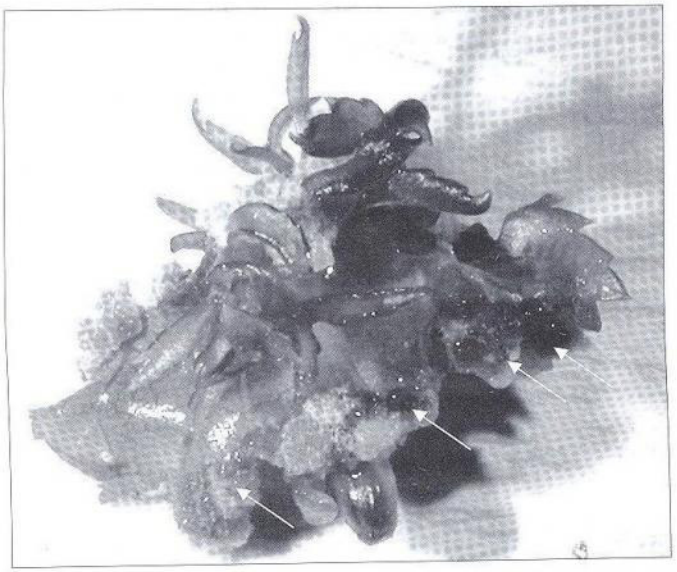

Figure 5. Shoot regeneration on explant of 'Royal Gala' after 14 days cultured in the light on regeneration medium contained 0.5 mg l^{-1} TDZ. New, developing shoot primordia are shown by arrows.

Table 4. Effects of N^9-ribosides on the shoot regeneration of 'Royal Gala' and 'M.26' after 7-week-long regeneration*

Applied concent-rations mg l^{-1} (μM)	'Royal Gala'				'M.26'			
	SN	R%	V%	OI	SN	R%	V%	OI
BAR								
0.5 (1.40)	1.38 a	13.0 a	1.6 a	0.16	2.0 ab	11.3 a	1.6 a	0.19
2.0 (5.60)	2.33 ab	64.8 bc	4.8 a	1.40	1.83 a	49.7 b	13.2 ab	0.67
3.5 (9.80)	3.51 b	88.2 c	13.1 a	2.64	1.79 a	48.0 b	6.4 ab	0.74
5.0 (14.00)	3.22 b	89.8 c	6.5 a	2.68	3.11 bc	59.7 bc	18.1 ab	1.29
6.5 (18.20)	3.47 b	81.6 c	4.8 a	2.66	3.22 c	76.5 bc	36.5 ab	1.29
8.0 (22.40)	3.22 b	52.9 b	3.2 a	1.60	2.78 abc	84.9 c	39.9 b	1.25
KINR								
0.5 (1.44)	0 a	0 a	0	0	0	0	0	0
2.0 (5.76)	1.0 a0	3.20 a	0	0.032	0	0	0	0
3.5 (10.08)	1.25 a	32.9 bc	0	0.41	0	0	0	0
5.0 (14.40)	1.25 a	13.1 ab	0	0.16	1.61 a	30.1 a	5.1 a	0.40
6.5 (18.72)	1.45 a	18.1 ab	0	0.26	1.55 a	33.2 a	5.1 a	0.44
8.0 (23.04)	1.60 a	41.2 c	0	0.66	1.28 a	28.3 a	3.4 a	0.32
TOPR								
0.5 (1.34)	1.00 a	3.2 a	0 a	0.03	1.33 a	4.8 a	0 a	0.06
2.0 (5.36)	1.55 ab	32.9 b	0 a	0.51	1.67 a	29.7 ab	1.6 ab	0.47
3.5 (9.38)	1.88 ab	39.8 b	0 a	0.75	1.93 a	44.6 b	8.1 b	0.70
5.0 (13.40)	2.15 ab	56.3 bc	3.2 a	1.14	1.44 a	41.3 b	4.8 ab	0.53
6.5 (17.42)	3.48 b	79.7 cd	3.2 a	2.66	1.64 a	46.4 b	1.6 ab	0.73
8.0 (21.44)	2.89 ab	93.3 d	3.2a	2.60	3.18 b	54.6 b	0 a	1.74
ZEAR								
0.5 (1.42)	1.0 a	3.2 a	0 a	0.03	1.33 a	5.0 a	0 a	0.07
2.0 (5.68)	1.33 a	29.7 b	3.2 a	0.35	1.18 a	17.9 ab	1.6 a	0.19
3.5 (9.94)	1.47 a	46.3 bc	9.8 a	0.68	1.19 a	26.3 ab	9.9 a	0.20
5.0 (14.20)	1.93 a	56.3 c	9.8 a	0.70	1.86 a	22.9 ab	6.5 a	0.30
6.5 (18.46)	2.44 a	64.7 cd	16.3 a	1.18	1.78 a	68.2 c	29.7 b	0.69
8.0 (22.82)	2.57 a	81.3 d	16.3 a	1.67	1.35 a	27.9 b	6.4 a	0.29

*The statistically homogeneous groups are indicated in every type of cytokinins in each apple cultivar by the same letter.

Application of TOP in the regeneration medium independently on cultivar was not so effective in the shoot regeneration from apple leaves than in the regeneration of other plant species, such as sugar beet (*Kubalákova & Strnad,* 1992). KIN and its riboside, furthermore isoprenoid cytokinins (ZEA, ZEAR) had low efficacy *(Figures 1 and 2, Tables 3–4).*

Comparing the organogenetic abilities of the two cultivars, it could be concluded, that 'Royal Gala' was a more responsible cultivar, since 3.5-fold higher SN and more than 4-fold higher OI was reached with this cultivar than with 'M.26'. Moreover, the similar developmental stage of shoots could be observed 3–5 days earlier than in 'M.26' *(Figures 3–4).* If explants of 'Royal Gala' were further-cultured, the SN increased, which might suggest higher organogenetic ability, too.

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