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# Light-induced alpha-chaconine and alpha-solanine accumulation in potato tubers (*Solanum tuberosum* L.) after harvest

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**ABSTRACT**

Tubers of all 31 Czech varieties listed in the National Book of Varieties of the Czech Republic in 1996 and three Slovakian varieties after harvest were exposed to the light (one week and 14 days) during the years 1996 and 1998 and the content of the two most important glycoalkaloids (α-chaconine and α-solanine) in them was measured. The reaction to light and to the effect of different duration of continuous lighting (one week and 14 days) differed among the varieties. In this way we simulated the conditions in many stores or supermarkets. High-performance liquid chromatography (HPLC) was used to determine α-chaconine and α-solanine in samples of tubers harvested after physiological maturation. Comparisons of the reaction of groups of varieties (early, medium early and medium late to late) showed that after exposure to one week of lighting (expressed in per cent), the increment in the content of $SGA_s$ decreased from the group of early to the medium late to late varieties. This was obviously associated with the total content of $SGA_s$ at the beginning of the experiment (prior to lighting). After leaving the tubers in the light for one week, the average content of α-solanine increased more than the content of α-chaconine in all the groups of varieties. After 14 days in light the average percentual growth of $SGA_s$ was the highest in the group of early varieties, then gradually lower from the medium early, and medium late to late varieties, to the lowest in the very early varieties. The different duration of lighting had a highly significant influence on the $SGA_s$ content in the respective groups divided according to the duration of the vegetative period. In all the 34 varieties the increase in the $SGA_s$ content in tubers after 14 days of lighting was approximately double (68.6 $mg.kg^{-1}$) the content in tubers kept in light for one week (33.1 $mg.kg^{-1}$). The effect of the conditions of the year of cultivation was also highly significant. The differences in the response of the varieties to light in terms of the increased $SGA_s$ content were significant (> 50 $mg.kg^{-1}$ fresh matter after one week and > 80 $mg.kg^{-1}$ after 14 days of light). The α-solanine:α-chaconine ratio in control tubers (kept in darkness) was 1:1.48, after one week in the light this ratio dropped to 1:1.44 and after another week of lighting it increased to 1:1.47.

**Keywords:** Czech potato varieties; $SGA_s$; α-solanine; α-chaconine; HPLC; response to light; duration of light; year of cultivation

During cultivation, harvesting, storage and processing of potato tubers until the inactivation of the respective enzymatic systems, metabolic processes, the products of which are the $SGA_s$, [$SGA_s$ = steroid glycoalkaloids (in chemical terms they are glycosides in which the steroid aglycones are bound to the oligo- or monosaccharides)] take place in the tissues of potato plants. An important factor affecting their content in the tubers may be physiological stress. Along with mechanical damage, the $SGA_s$ content in tubers is largely affected by light. Light induces and influences $SGA_s$ production along with other independent processes, such as synthesis of chlorophyll pigments (Percival 1999a). The sensitivity of the $SGA_s$ synthesis to light is based on the variety, physiological maturity and size of the tubers (Maine et al. 1988, Kozukue and Mizuno 1990).

Light intensity pays a very important role. For instance, Percival and Dixon (1996) investigated the increasing content of $SGA_s$ from a zero light exposure (darkness) to 500 $\mu mol.m^{-2}.s^{-1}$ of PAR for 96 hours and found that the $SGA_s$ contents increased in all the six potato genotypes included in the experiment, this increase being highly significant ($P < 0.01$). It was interesting that a further increase in the light intensity to ≥ 750 $\mu mol.m^{-2}.s^{-1}$ PAR lead to a reduced concentration of $SGA_s$ that decreased even more in 6 varieties when the light exposure was 1000 $\mu mol.m^{-2}.s^{-1}$ PAR and in 3 varieties when the light intensity was 1500 $\mu mol.m^{-2}.s^{-1}$ PAR.

The increase in $SGA_s$ content was much more dramatic after a 6-hour exposure to bright sunlight (35–50 thousand lux) when the solanidine glycoside content in the immediately harvested tubers increased from 50 $mg.kg^{-1}$ to > 200 $mg.kg^{-1}$ (Baerug 1962). A longer exposure (72 hours of intensive sunlight) resulted in a $SGA_s$ content of more than 450 $mg.kg^{-1}$ (Zitnak 1961, 1977).

The objective of the present study was to investigate the effect of light on the content of the two most important potato glycoalkaloids (α-chaconine and α-solanine) in tubers after harvest and the effect of a different duration of light on the final content. Individual varieties, or groups of varieties of the same vegetation period, were used for the investigations.

## MATERIAL AND METHODS

In field experiments carried out in 1996–1998 we planted the entire set of 31 Czech varieties (and 3 Slovakian

Table 1. $SGA_s$ content in $mg.kg^{-1}$ of fresh weight of raw unpeeled tubers of Czech cultivars (the 1996–1998 average)

| Group of cultivars | Number of cultivars | α-chaconine | | α-solanine | | Total $SGA_S$ | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ |
| Very early | 5 | 50.82 | 12.99 | 39.41 | 9.49 | 90.23 | 22.09 |
| Early | 11 | 46.55 | 15.88 | 28.95 | 12.68 | 75.50 | 25.59 |
| Medium early | 8 | 49.44 | 27.75 | 34.60 | 27.77 | 84.04 | 54.80 |
| Medium late to late | 10 | 74.08 | 47.13 | 49.21 | 35.11 | 123.29 | 81.10 |

Table 2. $SGA_s$ content in $mg.kg^{-1}$ of fresh weight in potato tubers lighted for one week (the 1996–1998 average)

| Group of cultivars | Number of cultivars | α-chaconine | | α-solanine | | Total $SGA_S$ | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ |
| Very early | 5 | 67.14 | 18.28 | 55.28 | 29.18 | 122.42 | 46.76 |
| Early | 11 | 65.88 | 32.16 | 43.40 | 19.75 | 109.28 | 48.88 |
| Medium early | 8 | 67.27 | 36.70 | 47.06 | 40.81 | 114.33 | 76.58 |
| Medium late to late | 10 | 94.39 | 64.58 | 63.95 | 43.57 | 158.34 | 105.61 |

varieties) listed in the National Book of Varieties of the Czech Republic in 1996. Based on the length of the vegetation period, we used 5 very early varieties, 9(+2) early varieties, 7(+1) medium early varieties and 10 medium late to late varieties. The treatment of the experiment during vegetation (cultural practices and nutrition) was briefly described in our previous work (Zrůst et al. 2000). The tubers were dug out manually, the soil was removed, then they were placed in a cooling locker and left for 14 days to respire at a temperature of about 18°C. After this time the temperature was gradually reduced to 4°C and the tubers were left in this temperature until they were used for further investigations (about one month).

The present study was focused on the effect of day and night light of low intensity (30–35 lx), the same as is used in small stores and/or supermarkets (where the lights are on throughout the night for security reasons), on $SGA_s$ production. We studied the content of $SGA_s$ in control tubers stored in darkness and in tubers intentionally exposed to continuous light (the tubers were placed on the floor in one layer in a room with windows, during the night lighted with a bulb) during one and two weeks.

We used 15 whole tubers of medium size with skin for the analyses (one from each harvested hill). The $SGA_s$ content was determined using high-performance liquid chromatography (HPLC) modified by Kobayashi et al.

Table 3. Analysis of variance of $SGA_s$ content in tubers exposed to light for one week

| Source of variability | *d.f.* | Sum of squares | *F*-values | Significance |
|---|---|---|---|---|
| Replication | 2 | 40.805 | 1.423 | |
| Earliness⁺ (A) | 3 | 14 370.591 | 334.060 | ** |
| Years (B) | 2 | 50 220.832 | 1 751.159 | ** |
| A × B | 6 | 36 174.533 | 420.459 | ** |
| Residue | 22 | 315.465 | | |
| Total | 35 | 101 122.225 | | |

⁺ variety groups according to maturity (earliness), ** significant at 0.01 probability level

| Significant differences | | | | | | | |
|---|---|---|---|---|---|---|---|
| Earliness⁺ | mean | Tukey | | years | mean | Tukey | |
| ML-L | 158.34 | 5% | 4.93 | 1996 | 164.52 | 5% | 3.86 |
| VE | 122.42 | 1% | 6.21 | 1997 | 135.95 | 1% | 4.98 |
| ME | 113.35 | | | 1998 | 74.97 | | |
| E | 106.49 | | | | | | |

⁺ variety groups according to maturity (earliness): VE = very early, E = early, ME = medium early, ML-L = medium late to late

Table 4. Analysis of variance of $SGA_s$ content in tubers exposed to light for two weeks

| Source of variability | *d.f.* | Sum of squares | *F*-values | Significance |
|---|---|---|---|---|
| Replication | 2 | 50.317 | 0.583 | |
| Earliness⁺ (A) | 3 | 27 369.656 | 211.246 | ** |
| Years (B) | 2 | 65 797.328 | 761.761 | ** |
| A × B | 6 | 43 928.985 | 169.528 | ** |
| Residue | 22 | 950.128 | | |
| Total | 35 | 138 096.415 | | |

| Significant differences | | | | | |
|---|---|---|---|---|---|
| Earliness⁺ | mean | Tukey | years | mean | Tukey |
| ML-L | 207.06 | 5% 8.55 | 1996 | 207.79 | 5% 6.70 |
| E | 144.87 | 1% 10.78 | 1997 | 166.41 | 1% 8.65 |
| VE | 142.86 | | 1998 | 103.79 | |
| ME | 142.52 | | | | |

(1989) and taking into consideration other experience (Kvasnička et al. 1994) too. The apparatuses and techniques used and the conditions of the analysis have been reported earlier (Zrůst 1997). The chromatographic Apex software was used to interpret the chromatograms. The method of the calibration curve was used for the quantification.

## RESULTS AND DISCUSSION

The total content of $SGA_s$ in tubers of Czech potato varieties kept in darkness (control tubers) gradually increased from the group of early tubers through the medium early variety to the medium late to late varieties. The average total content of $SGA_s$ in the very early varieties was higher than in the medium early ones (Table 1). After the tubers were kept for one week in the laboratory in light and in an average temperature of 20°C and additional lighting with a bulb in the night, $SGA_s$ contents in the tubers of the individual groups of varieties increased (Table 2).

The increment in the $SGA_s$ content after one-week exposure to light decreased in the order from the early to the medium late to late varieties. The percentual increase in the $SGA_s$ content in the very early and medium early varieties was virtually the same and was apparently associated with the total content of $SGA_s$ at the beginning of the experiment (in the control tubers kept in darkness). After one-week exposure to light the differences in the $SGA_s$ contents among the respective varieties were highly significant (Table 3).

After one-week exposure of the tubers to light the average content of α-solanine increased more than the α-chaconine content in all the groups of varieties. The α-solanine:α-chaconine ratio decreased from 1:1.48 in tubers kept in darkness to 1:1.44.

When we compared the average increase in the $SGA_s$ content of tubers in all the 34 varieties kept in light for 14 days and 7 days, the content of the former was approximately double (i.e. 68.6 $mg.kg^{-1}$ and 33.1 $mg.kg^{-1}$, respectively) (Table 4, Figure 1). After 14 days the average percentual SGAs growth was the highest in the group of early varieties. Percentual difference in increase in $SGA_s$ content between the first and the second week of lighting was also highest – it reached nearly 50%. This increase gradually decreased from the medium early to the medium late to late varieties and was the lowest in the very early varieties. Difference between the both weeks of lighting in growth of $SGA_s$ content was the lowest in very early varieties (22.6%) less than half compared to the group of early varieties. The weight increment was the highest in the medium late to late varieties and then gradually decreased in the groups of early, medium early varieties to the lowest in the very early varieties. The differences in the $SGA_s$ content after 14 days of exposure were highly significant only in the medium late to late varieties. In the other groups the $SGA_s$ averages ranged around 2.35 $mg.kg^{-1}$ (Table 5). The α-solanine:α-chaconine ratio after the second week of lighting increased to 1:1.47, however, it was lower than in the control tubers (stored in darkness).

Percival (1999b) drew the same conclusions when he evaluated tubers of 5 varieties exposed to a 15-day period of continuous radiation of an intensity of 250 $\mu mol.m^{-2}.s^{-1}$ photosynthetically active radiation (PAR). This exposure decreased the α-chaconine:α-solanine ratio but did not significantly affect the chlorophyll *a*:*b* ratio (Percival 1999a).

In our experiments the effect of the different period of lighting on the $SGA_s$ content was highly significant (Table 6). The $SGA_s$ content in tubers of the entire group of 34 varieties exposed to 14 days of light was 34.18 $mg.kg^{-1}$ of fresh matter higher. In percent the $SGA_s$ content in these tubers was 27.3% higher than in tubers exposed to light for one week.

In this respect the results of experiments carried out by Frydecka-Mazurczyk (1998) in 1992–1994 are important. She applied various light intensities and found that the

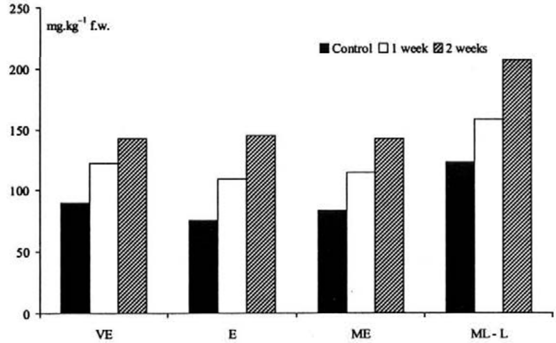


Figure 1. Increase in total $SGA_s$ content after one week and two weeks of tuber lighting in individual maturity groups (Legend see Table 1)

$SGA_s$ content increased under a higher intensity of light. She also found that the $SGA_s$ synthesis was stimulated in tubers exposed to light of such a low intensity as is used in the stores (between 3–25 $\mu mol.m^{-2}.s^{-1}$) or under fluorescent lighting (3–15 $\mu mol.m^{-2}.s^{-1}$). In the present study we did not study the intensity of light.

Our results, as well as those from abroad, confirmed that it is important to cover up the tubers properly during cultivation (to prevent greening), it is recommended not to leave the tubers in the light during harvesting, and the same is recommended for temporary storage and post-harvest treatment. Frydecka-Mazurczyk (1998) proved that transporting the tubers in suitable packaging – sacks – under a reduced intensity of light is important, too. Tubers should not be also exposed to the light when sold in shops and on fairs.

Table 5. $SGA_s$ content in $mg.kg^{-1}$ in tubers lighted for two weeks (the 1996–1998 average

| Group of cultivars | Number of cultivars | α-chaconine | | α-solanine | | Total $SGA_S$ | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ | $\bar{x}$ | $s_{\bar{x}}$ |
| Very early | 5 | 77.48 | 49.77 | 65.38 | 45.81 | 142.86 | 94.87 |
| Early | 11 | 85.38 | 61.46 | 59.59 | 41.91 | 144.97 | 101.72 |
| Medium early | 8 | 85.26 | 54.80 | 57.26 | 49.17 | 142.52 | 102.70 |
| Medium late to late | 10 | 127.63 | 93.60 | 79.43 | 59.86 | 207.06 | 150.45 |

Table 6. Analysis of variance of $SGA_s$ content in tubers exposed to light

| Source of variability | *d.f.* | Sum of squares | *F*-values | Significance |
|---|---|---|---|---|
| Replication | 2 | 24.360 | 0.421 | |
| Earliness + (A) | 3 | 39 747.778 | 457.435 | ** |
| Years (B) | 2 | 115 267.731 | 1 989.829 | ** |
| Exposure duration (C) | 1 | 21 030.612 | 726.089 | ** |
| A × B | 6 | 79 701.353 | 458.619 | ** |
| A × C | 3 | 1 992.469 | 22.930 | ** |
| B × C | 2 | 750.429 | 12.954 | ** |
| A × B × C | 6 | 402.165 | 2.314 | * |
| Residue | 46 | 1 332.354 | | |
| Total | 71 | 260 249.252 | | |

+ variety groups according to maturity (earliness), ** significant at 0.01 probability level, * significant at 0.05 probability level

| Significant differences | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Earliness+ | mean | Tukey | | years | mean | Tukey | | exposure | mean | Scheffe | |
| ML-L | 182.70 | 5% | 4.79 | 1996 | 186.15 | 5% | 3.77 | two weeks | 159.33 | 5% | 2.54 |
| VE | 132.64 | 1% | 5.92 | 1997 | 151.18 | 1% | 4.77 | one week | 125.15 | 1% | 3.37 |
| ME | 127.93 | | | 1998 | 89.38 | | | | | | |
| E | 125.68 | | | | | | | | | | |

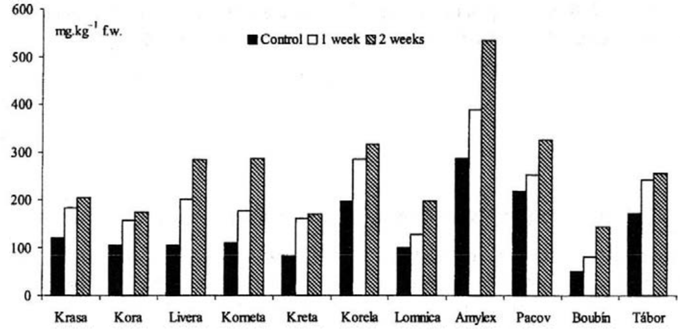


Figure 2. Varieties with increased response to light

In our experiments the effect of the year of cultivation was highly significant (Table 6), the order being from 1996 (the highest content) to 1998 (the lowest, only 48% of $SGA_s$ compared to 1996). The reasons for the increased $SGA_s$ content (i.e. higher temperatures, drought, above-average period of sunshine during vegetation) have been described earlier (Zrůst 1997).

In the total analysis of variance incorporating both periods of lighting the difference in the $SGA_s$ content between the medium late to late varieties and the other groups of varieties were highly significant, and the content in the very early varieties was highly significantly higher than in the early varieties (Table 6). The differences among the individual varieties were highly significant because $SGA_s$ synthesis was their response to the duration of lighting. The varieties that responded to light markedly in that they increased the $SGA_s$ increments are shown in the graph (Figure 2).

For the graph we selected varieties where the $SGA_s$ content increases by more than 50 $mg.kg^{-1}$ after one week of lighting, after 14 days the content of α-chaconine and α-solanine increased by more than 80 $mg.kg^{-1}$. Among the varieties some exceptions occurred that did not meet the two defined criteria. In a 3-year average the variety Kora did not exceed 70 $mg.kg^{-1}$ in the second week. Also in a 3-year average the varieties Lomnica, Pacov and Boubín synthesised about 30 $mg.kg^{-1}$ of $SGA_s$ in the first week of lighting, while the two former varieties synthesised more than 100 $mg.kg^{-1}$ of $SGA_s$ within the next week.

The variety Kreta, on the contrary, met both criteria in the very first week of lighting, the 3-year average increase in the $SGA_s$ content was 88.69 $mg.kg^{-1}$, during the second week it synthesised only 10 $mg.kg^{-1}$ of fresh matter. Also the varieties Tábor and Krasa increased the content of $SGA_s$ only very little after the second week (i.e. 13 and 21 $mg.kg^{-1}$, respectively).

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## ABSTRAKT

**Akumulace α-chaconinu a α-solaninu v hlízách bramboru (*Solanum tuberosum* L.) vyvolaná vlivem světla po sklizni**

Sklizené hlízy všech 31 českých odrůd registrovaných ve Státní odrůdové knize ČR v r. 1996 a tří odrůd slovenských byly v letech 1996 až 1998 vystaveny světlu (týden a 14 dnů) a poté v nich změřen obsah dvou nejvýznamnějších glykoalkaloidů (α-chaconin a α-solanin). Tímto postupem byly simulovány podmínky vyskytující se v řadě obchodů, případně supermarketů. V reakci na světlo i v působení rozdílné délky trvalého osvětlení se projevily odrůdové rozdíly. Pro stanovení α-chaconinu a α-solaninu ze vzorků hlíz sklizených po fyziologickém dozrání se použilo vysokoúčinné kapalinové chromatografie (HPLC). Celkový obsah $SGA_s$ v hlízách českých odrůd ponechaných ve tmě (kontrolní hlízy) se zvyšoval postupně od skupiny raných přes polorané ke skupině odrůd polopozdních až pozdních. Velmi rané odrůdy měly v průměru celkový obsah $SGA_s$ vyšší než odrůdy polorané (tab. 1). Po jednotýdenním ponechání hlíz v laboratoři na světle (v jedné vrstvě na podlaze 2–3 m od okna) při průměrné teplotě 20 °C a přisvětlováním žárovkou v noci (na úrovni hlíz naměřeno 30–35 lx) se zvýšily obsahy $SGA_s$ v hlízách jednotlivých skupin odrůd (tab. 2). Přírůstek obsahu $SGA_s$ po vystavení jednotýdennímu světlu vyjádřený v % klesal ve směru od raných po polopozdní až pozdní odrůdy. Zřejmě to souviselo s celkovým obsahem $SGA_s$ na počátku pokusu. Jednotlivé skupiny odrůd měly po týdenním osvícení navzájem vysoce významně rozdílné obsahy $SGA_s$ (tab. 3). Obsah α-solaninu se v průměru všech skupin odrůd zvýšil po týdenním ponechání hlíz na světle více než obsah α-chaconinu. V průměru ze všech 34 odrůd byl přírůstek celkových $SGA_s$ za 14 dní u hlíz ponechaných na světle přibližně dvojnásobný (68,6 $mg.kg^{-1}$) oproti hlízám osvětleným jeden týden (33,1 $mg.kg^{-1}$). Na obr. 1 jsou vyjádřeny přírůstky celkových $SGA_s$ po týdnu a 14 dnech osvětlení v jednotlivých skupinách odrůd rozlišených podle délky vegetace. Ve skupinách se vysoce významně odlišily obsahem $SGA_s$ po 14denním osvitu pouze polopozdní až pozdní odrůdy (tab. 4). V ostatních skupinách se průměry $SGA_s$ pohybovaly v úzkém rozmezí kolem 2,35 $mg.kg^{-1}$ (tab. 5). V celkové analýze variance zahrnující obě délky osvětlení (tab. 6) měly vysoce průkazný rozdíl obsahu $SGA_s$ polopozdní až pozdní odrůdy oproti ostatním skupinám odrůd a velmi rané odrůdy měly rovněž vysoce významně vyšší obsah oproti raným odrůdám. Vliv ročníku byl vysoce významný (tab. 6). Odrůdy s výraznou reakcí na světlo zvýšenými přírůstky $SGA_s$ (> 50 $mg.kg^{-1}$ č. h. po týdnu a > 80 $mg.kg^{-1}$ č. h. po 14 dnech působení světla) jsou znázorněny na obr. 2. Poměr α-solaninu ku α-chaconinu u hlíz kontrolních (ponechaných ve tmě) byl 1:1,48, po týdenním ponechání hlíz na světle se tento poměr snížil na 1:1,44, za další týden osvětlení hlíz se zvýšil na 1:1,47. Jak naše, tak citované zahraniční výsledky přesvědčují o důležitosti správného zahrnutí hlíz při kultivaci (zabránění zelenání hlíz) a doporučení neponechávat hlízy při sklizni zbytečně na světle, což platí i pro přechodné skládky a posklizňovou úpravu. Rovněž expedice hlíz ve vhodných obalech, resp. pytlích a zabránění zbytečné expozici hlíz světlem při prodeji v obchodech a na tržištích jsou z tohoto pohledu důležité.

**Klíčová slova:** české odrůdy bramboru; steroidní glykoalkaloidy; α-solanin; α-chaconin; HPLC; reakce na světlo; délka světla; ročník pěstování

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# Effects of various ways of pre-plant soil preparation on potato tuber yields and soil physical properties and soil temperature

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**ABSTRACT**

In 1997–2000 based on exact field trials, an effect of autumn (ploughing or frost damaged intercrop sowing) and spring soil cultivation technologies (conventional technology – loosening before planting, cultivation and post-plant herbicide treatment or de-stoning technology – furrowing and stone and clod separation before planting and post-plant herbicide treatment) on potato tuber yields, soil hydrophysical parameters (measured during growing period) and soil temperature (daily measured at 7 a.m. and 2 p.m. in depths of 50 and 150 mm) was evaluated. Statistically significantly higher tuber yields were found in technologies with de-stoning compared to technologies with conventional spring soil preparation, on average about 3.73 $t.ha^{-1}$ (in combination with ploughing system) and 9.04 $t.ha^{-1}$ (ploughless technology), lower penetrometric resistance after planting and before harvest and associated lower bulk density of soil (difference on average of 0.03 $g.cm^{-3}$). Porosity values were statistically significantly higher in both technologies with de-stoning (about 1.34% on average of all measurements). On the contrary, volume and relative soil moisture and maximum water capacity were statistically significantly lower in technologies with de-stoning compared to conventional technologies, namely on average about 1.10, 3.45 and 2.83%, respectively. Technologies with de-stoning generally reached lower morning soil temperatures in topsoil depth of 50 mm during the growing period, however, afternoon temperatures in depth of 50 mm were unambiguously about 0.40–0.75°C higher than in conventional technologies (in temperature sum during growing period in variants with autumn ploughing and spring de-stoning about 58.4°C compared to technology with autumn ploughing and conventional spring soil preparation and in autumn ploughless system and spring de-stoning about 39.9°C, in the depth of 150 mm it was only true for combination of ploughing variants).

**Keywords:** potatoes; soil preparation; de-stoning technology; soil physical characteristics; soil temperatures

At the present, development of alternative technologies of soil preparation and crop establishment occur in agricultural crops. In potatoes it is true for growing technology in de-stoned beds. In the Czech Republic, almost all agricultural enterprises directed to potato production have passed to this technology during last 8 years. A change in technology particularly affects spring soil preparation, but also autumn soil preparation could be inferior to this change. Besides the effect of removal of average 60% of stones from the ridge and also reduced mechanical tuber damage during harvest and other handlings, it is presupposed that the technology has an essential effect on soil physical characteristics. The effect has not been sufficiently studied yet and the work provides original findings.

Alternative ways of potato crop establishment concern autumn soil preparation (shallow cultivation or no-ploughing), also spring soil preparation (de-stoning). Vos and Kooistra (1994) assessed five different systems of soil cultivation. They found similar potato yields in conventional system and system with long-term (18 years) shallow soil cultivation, however, minimizing system had more favourable effect on soil physical characteristics due to stimulation of soil fauna activity. In the USA, Westermann and Sojka (1996) evaluated technology of deep ploughing according to hypothesis that inter-row subsoiling and local nitrogen placement could affect tuber yield and nutrition status of plants; however, it was found that autumn ploughing did not influence concentration and nutrient uptake, tuber yield and quality. Fér and Čepl (1997) refer to a short history of development and applying technology of de-stoning in the Czech Republic, principals and technical solution. Technology has an origin in Scotland; from there it was spread all over the Europe including Nordic states (Andersson 1997).

In the literature there are not many data on effect of mechanical practices on change in soil physical characteristics in potato growing. Most works are reduced to water stress or limit values of soil water content generally or with respect to irrigation (Costa et al. 1997) in the field of water regime. Nazarenko et al. (1991) studied effect of different soil cultivation (passive and active bodies) on soil bulk density. In soil cultivation with active apparatus, soil was less compacted in the whole growing period and had more favourable characteristics of air regime; it was exhibited by positive effect on tuber, dry matter and starch yield. De-stoning technology is particularly represented by values of cultivation practice effectiveness on soil stone content and performance of individual set types (Andersson 1997). Diviš and Špína (1998) aimed at evaluation of soil physical characteristics and found that higher tuber yield was reached in applying technology of de-stoning and soil kept a character of very loose soil during the whole growing period.

Table 1. Mean temperatures and rainfall sum in growing periods of 1997–2000 on the site of exact field trials

| Observed parameter | April | May | June | July | August | September | Sum/Mean |
|---|---|---|---|---|---|---|---|
| | | | | Rainfall (mm) | | | |
| Mean* | 42.5 | 76.3 | 91.4 | 80.9 | 86.6 | 48.2 | 425.9 |
| 1997 | 52.2 | 72.2 | 55.0 | 199.0 | 45.6 | 12.4 | 436.4 |
| 1998 | 21.7 | 71.6 | 139.7 | 168.8 | 43.7 | 100.5 | 546.0 |
| 1999 | 35.2 | 59.1 | 93.3 | 133.7 | 50.3 | 34.0 | 405.6 |
| 2000 | 30.1 | 46.8 | 64.0 | 115.8 | 55.3 | 41.6 | 353.6 |
| | | | | Temperature (°C) | | | |
| Mean* | 7.3 | 11.6 | 15.2 | 16.5 | 16.4 | 12.3 | 13.2 |
| 1997 | 4.7 | 13.8 | 17.2 | 16.8 | 19.2 | 13.4 | 14.2 |
| 1998 | 8.0 | 11.4 | 15.6 | 16.1 | 16.2 | 11.1 | 13.1 |
| 1999 | 7.5 | 11.8 | 13.7 | 18.3 | 16.0 | 15.1 | 13.7 |
| 2000 | 9.9 | 13.4 | 15.7 | 14.5 | 17.6 | 11.6 | 13.8 |

* mean over 70 years

## MATERIAL AND METHODS

Exact field trials were established according to the principles of experimental technique in 1997–2000 at the Research Station Valečov of Potato Research Institute Havlíčkův Brod. The station is situated in the above sea level of 450 m, soil is medium-heavy with loam to sand-loam topsoil. Weather conditions are present in Table 1.

Following ways of autumn and spring soil preparation for potatoes were variants of the trial.

+PCT = conventional technology of growing with ploughing (autumn – skimming, ploughing on full depth of topsoil, spring – smoothing, loosening with cultivator, planting, prior to plant emergence – harrowing, ridging, herbicide)
+PDS = technology of de-stoning with ploughing (autumn – skimming, ploughing on full depth of topsoil, spring – furrowing, clod and stone separation, planting, prior to plant emergence – herbicide)
–PCT = conventional technology without ploughing (autumn – sowing of frost damaged intercrop white mustard, spring – crushing of mustard residue, loosening with cultivator, planting, prior to plant emergence – harrowing, ridging, herbicide)
–PDS = technology of de-stoning without ploughing (autumn – sowing of frost damaged intercrop white mustard, spring – crushing of mustard residue, furrowing, clod and stone separation, planting, prior to plant emergence – herbicide)

The area of trial plots was 180 $m^2$ (4 rows × 60 m), harvest plot was 22.5 $m^2$. Each variant had four replications. Early table potato variety Karin was used for the trial.

During the growing period soil sampling was carried out using Kopecky rollers in all the variants and replications (total eight samplings). According to the modified method after Novák (1947, cit. Drbal 1986) following hydrophysical parameters were measured – soil bulk density ($g.cm^{-3}$), soil moisture (% volume), relative soil moisture (% relative), porosity (% volume), maximum capillary water capacity (% volume). Measurement of soil resistance (MPa) with digital penetrometer was performed in 1998–2000 after planting and before harvest in the whole topsoil profile. Twice a day (at 7 a.m. and 2 p.m.) soil temperatures were measured in two depths of topsoil (50 mm and 150 mm) in the whole growing period. Weight of tubers from harvest plots was determined in the period of physiological maturity. Statistical evaluation was done using variance analysis, significance dif-

Table 2. Effect of different soil cultivation on tuber yield

| Experimental year | Variant | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | +PCT | +PDS | –PCT | –PDS | *F*-test | $P_{0.05}$ | $P_{0.01}$ |
| 1997 | 34.86 | 38.59 | 33.85 | 42.89 | 9.79** | 6.42 | 9.22 |
| 1998 | 51.56 | 62.67 | 40.22 | 53.33 | 43.72** | 6.83 | 7.64 |
| 1999 | 32.89 | 47.78 | 39.78 | 43.45 | 114.26** | 2.89 | 4.15 |
| 2000 | 44.11 | 45.33 | 43.89 | 40.00 | 2.42 | 7.28 | 10.44 |
| Mean | 40.86 | 48.59 | 39.44 | 44.92 | 44.17** | 2.43 | 3.06 |
| Statistical values for years | | | | | 29.16** | 5.15 | 7.05 |

Table 3. Effect of different soil cultivation on bulk density of soil ($g.cm^{-3}$)

| Variant | DAP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 34 | 48 | 69 | 83 | 97 | 111 | 122 |
| +PCT | 1.25 | 1.24 | 1.24 | 1.26 | 1.25 | 1.27 | 1.25 | 1.25 |
| +PDS | 1.22 | 1.19 | 1.20 | 1.21 | 1.21 | 1.25 | 1.22 | 1.20 |
| –PCT | 1.25 | 1.24 | 1.18 | 1.16 | 1.22 | 1.24 | 1.22 | 1.21 |
| –PDS | 1.24 | 1.22 | 1.15 | 1.16 | 1.17 | 1.22 | 1.22 | 1.22 |

DAP – days after planting

Table 4. Effect of different soil cultivation on porosity (%)

| Variant | DAP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 34 | 48 | 69 | 83 | 97 | 111 | 122 |
| +PCT | 51.9 | 52.4 | 52.2 | 51.9 | 52.1 | 51.5 | 49.2 | 52.2 |
| +PDS | 53.4 | 54.3 | 53.8 | 53.3 | 53.7 | 52.5 | 53.0 | 53.7 |
| –PCT | 51.8 | 52.1 | 54.8 | 54.8 | 53.2 | 52.7 | 53.0 | 53.2 |
| –PDS | 52.7 | 53.3 | 55.9 | 55.6 | 55.4 | 53.4 | 53.4 | 53.2 |

Table 5. Effect of different soil cultivation on relative soil moisture (%)

| Variant | DAP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 34 | 48 | 69 | 83 | 97 | 111 | 122 |
| +PCT | 33.7 | 39.1 | 43.7 | 43.8 | 40.0 | 42.8 | 35.8 | 33.4 |
| +PDS | 32.9 | 36.2 | 38.5 | 39.7 | 36.9 | 39.9 | 27.4 | 30.8 |
| –PCT | 33.0 | 41.9 | 39.6 | 35.3 | 40.6 | 41.3 | 28.4 | 36.4 |
| –PDS | 32.4 | 37.5 | 35.7 | 36.0 | 34.8 | 36.8 | 25.7 | 32.3 |

ferences according to Tukey and Scheffe. All mentioned results are mean values over four experimental years.

## RESULTS AND DISCUSSION

Results of tuber yield are present in Table 2. Statistically significantly higher yield on average of years compared to other variants was found in de-stoning technology with ploughing (+PDS) 48.6 $t.ha^{-1}$, further there was followed statistically highly significant yield for all other variants of no-ploughing technology of de-stoning (–PDS) 44.9 $t.ha^{-1}$. Significant differences were not observed in conventional ploughing technology (+PCT) with 40.9 $t.ha^{-1}$ and conventional no-ploughing technology (–PCT) with 39.4 $t.ha^{-1}$. Yield results confirm work of Fér et al. (2000) and Diviš and Špína (1998).

Soil penetrometric resistance measured after planting (Figure 1) was lowest in +PDS technology in topsoil profile. Due to proper topsoil sifting with separator, soil re-

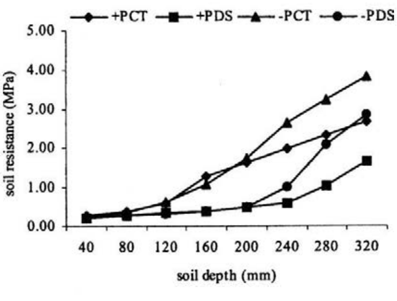


Figure 1. Penetrometric soil resistance after planting

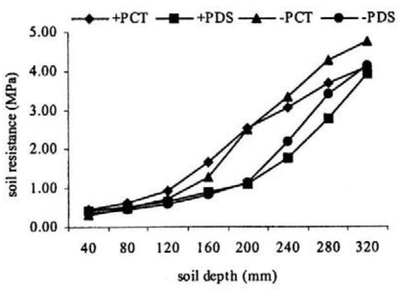


Figure 2. Penetrometric soil resistance before harvest

Table 6. Effect of different soil cultivation on soil moisture (% volume)

| Variant | DAP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 34 | 48 | 69 | 83 | 97 | 111 | 122 |
| +PCT | 17.5 | 20.4 | 22.6 | 22.4 | 20.9 | 21.5 | 14.9 | 17.3 |
| +PDS | 17.6 | 19.6 | 20.7 | 20.9 | 19.8 | 20.3 | 14.6 | 16.5 |
| −PCT | 17.1 | 21.4 | 21.6 | 19.3 | 21.3 | 21.4 | 14.9 | 19.2 |
| −PDS | 17.1 | 20.0 | 19.9 | 19.9 | 19.3 | 19.2 | 13.7 | 17.0 |

Table 7. Effect of different soil cultivation on maximum water capacity (% volume)

| Variant | DAP | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 34 | 48 | 69 | 83 | 97 | 111 | 122 |
| +PCT | 38.63 | 36.24 | 35.37 | 34.73 | 35.16 | 34.84 | 34.33 | 31.76 |
| +PDS | 36.79 | 33.45 | 31.85 | 32.25 | 32.78 | 33.40 | 32.65 | 29.49 |
| −PCT | 41.09 | 38.35 | 35.43 | 35.63 | 36.59 | 36.01 | 36.34 | 33.06 |
| −PDS | 37.91 | 34.89 | 32.25 | 32.28 | 32.13 | 34.04 | 32.90 | 29.29 |

sistance in both variants with de-stoning was in dependence on measurement depth always generally lower than in variants with conventional technology. This finding was also fully confirmed in soil resistance measurement before harvest (Figure 2) in the framework of variants of spring soil preparation (de-stoning and conventional preparation), where elimination of tractor trips leaving out cultivation practices in de-stoning played a role.

Bulk density of soil (Tables 3 and 8) periodically measured during growing period as criterion of soil compaction, was statistically significantly lower in both variants with de-stoning ($F = 17.19^{**}$) compared to both variants of conventional soil preparation. The same conclusions are true as in evaluation of penetrometric soil resistance. Bulk density was not influenced by year effects ($F = 1.96$).

Table 8. Statistical evaluation of soil hydrophysical parameters

| Parameter | Unit | Variant | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|---|
| | | +PCT | +PDS | −PCT | −PDS | variant factor | | |
| | | mean over time series | | | | $F$-test | significant difference | |
| | | | | | | | $P_{0.05}$ | $P_{0.01}$ |
| Bulk density | g.cm$^{-3}$ | 1.25 | 1.21 | 1.22 | 1.20 | 17.191$^{**}$ | 0.019 | 0.024 |
| Volume moisture | % vol. | 19.70 | 18.76 | 19.51 | 18.26 | 8.866$^{**}$ | 0.827 | 1.012 |
| Relative moisture | % rel. | 39.03 | 35.32 | 37.07 | 33.88 | 11.326$^{**}$ | 2.432 | 2.973 |
| Porosity | % vol. | 51.68 | 53.46 | 53.23 | 54.10 | 13.483$^{**}$ | 1.027 | 1.255 |
| Max. capillary capacity | % vol. | 35.13 | 32.83 | 36.56 | 33.21 | 42.201$^{**}$ | 0.985 | 1.205 |

| Parameter | Unit | Year factor | | |
|---|---|---|---|---|
| | | $F$-test | significant difference | |
| | | | $P_{0.05}$ | $P_{0.01}$ |
| Bulk density | g.cm$^{-3}$ | 1.955 | 0.040 | 0.049 |
| Volume moisture | % vol. | 24.540$^{**}$ | 3.400 | 4.165 |
| Relative moisture | % rel. | 21.171$^{**}$ | 7.247 | 8.877 |
| Porosity | % vol. | 0.718 | 1.611 | 1.974 |
| Max. capillary capacity | % vol. | 11.916$^{**}$ | 2.159 | 2.645 |

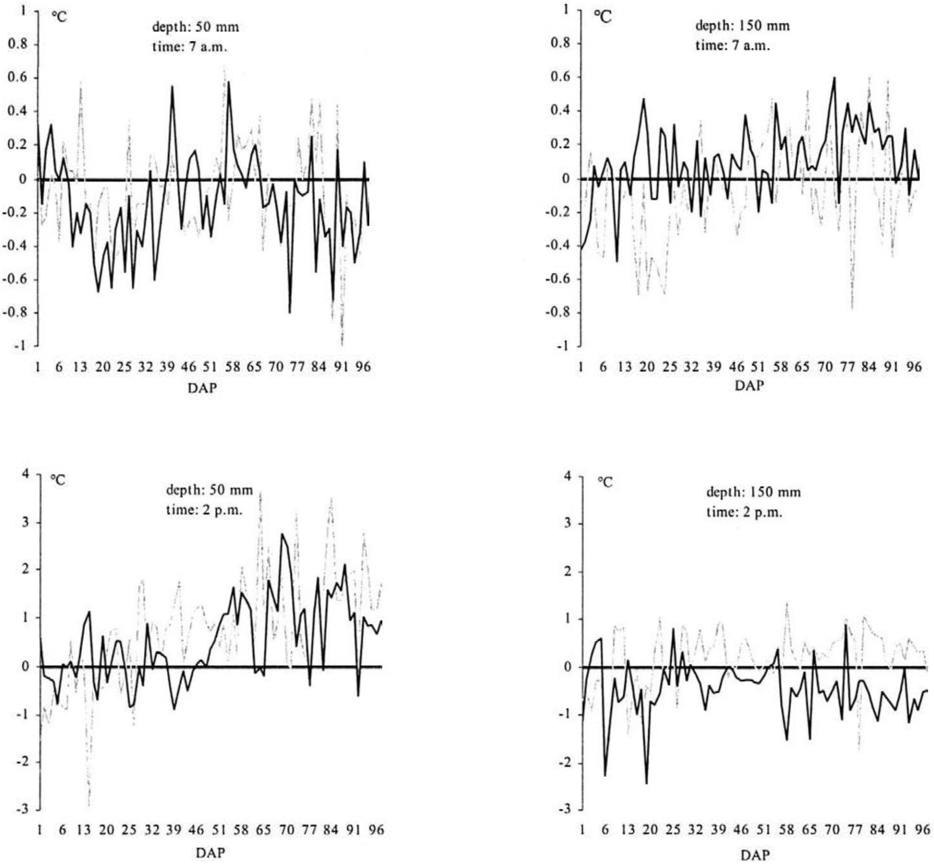


Figure 3. Differences in soil temperatures between variants during growing period [DAP = days after planting, — diference (+PDS) – (+PCT), ▬ diference (–PDS) – (–PCT)]

Porosity, i.e. portion of pores from soil volume (Tables 4 and 8) was statistically significantly higher during the growing period in both variants – technology of spring soil preparation with de-stoning ($F = 13.48^{**}$) with the fact that for no-ploughing in –PDS technology portion of pores increased compared to +PDS variant. Porosity was not statistically significantly influenced by the year ($F = 0.17$).

Results of all soil compaction characteristics explain positive effect of de-stoning technology on yield parameters. This finding is in accordance with conclusions of Vos and Kooistra (1994), who found that potatoes respond most sensitively to soil compaction of all crops.

Bulk density of soil (Tables 5 and 8) as a parameter of whole water content in soil was statistically significantly higher in both variants with conventional spring soil preparation than in technology of de-stoning ($F = 8.87^{**}$) in the whole growing period and from the results it is concluded that no-ploughing was exhibited by further reducing of the parameter. Year effect was stronger than the effect of soil cultivation ($F = 24.54^{**}$), namely in the year 1998 due to rainfall sum higher about 28.2% than long-term mean (Table 1).

Relative moisture (Tables 6 and 8), which informs on water ratio to pore volume, could be characterized similarly as the previous parameter. Effect of spring soil cultivation was highly significant in sense of significant differences between variants +PCT and +PDS on one side and –PCT and –PDS on the other side, i.e. between technologies of spring soil preparation with higher values in de-stoning. Year had stronger effect than variants of soil cultivation ($F = 21.17^{**}$).

Values of maximum water capacity (Tables 7 and 8), which represent ability of soil to retain water for vegetation needs, were influenced by ways of soil cultivation ($F = 42.20^{**}$), but also by year ($F = 11.92^{**}$). Statistically significant differences were found only between +PCT and –PCT variants to +PDS and –PDS variants, where variants with conventional spring soil preparation (irrespective of autumn preparation) had always higher measured values in the whole growing period.

Generally, we can conclude with respect to characteristics of soil physical properties that observed variants affected them statistically significantly only from the viewpoint of spring soil preparation, factor of autumn soil preparation had no significant effect. All parameters characterizing soil compaction were more favourable for technologies with de-stoning; it was inverse in the characteristics of water regime.

Results of soil temperature measurements are given in Figure 3. There are differences of temperatures between technology variants of spring soil preparation within the same technology of autumn soil preparation, since it was found that autumn soil preparations had minimum and neglectable effect on soil temperatures in the growing period.

Variants with de-stoning had lower temperature than variants with conventional spring soil preparation in the morning measurement shallow under soil surface (50 mm). In comparison of +PCT and +PDS variants difference in sum of temperatures during the growing period is 6.5°C in favour to +PCT variant and in comparison of –PCT and –PDS variants even 11.2°C. In the topsoil depth of 150 mm only minimum difference of 4.6°C was recorded between +PCT and +PDS in favour to +PCT and in comparison of temperatures in –PCT and –PDS variants there were found higher values in –PDS, namely about 7.8°C. Afternoon temperature measurement in the depth of 50 mm had unambiguous trend of higher temperatures in variants of de-stoning compared to conventional spring soil preparation irrespective of way of autumn soil preparation. Differences in sum of temperatures were 58.4°C between two variants with ploughing and 39.9°C without ploughing. Higher differences firstly occurred in the period of tuber setting. In the topsoil depth of 150 mm, this finding was only true within +PCT and +PDS variants (difference of 11.8°C in favour to +PDS), however difference of 35.2°C in favour to –PCT was detected in –PCT and –PDS variants.

## CONCLUSION

Based on the results of exact field trials, the work evaluated an effect of technologies of autumn and spring soil cultivation on potato tuber yields and soil hydrophysical parameters. Statistically significantly higher tuber yields were found among variants with de-stoning and conventional spring soil preparation irrespective of way of autumn soil preparation. Lower penetrometric resistance was recorded for technologies with de-stoning after planting and before harvest and also associated lower bulk density of soil was found. Porosity values were also statistically significantly higher in those technologies. On the contrary, volume and relative soil moisture and maximum water capacity were significantly lower in both variants of spring soil cultivation with de-stoning rather than in conventional technology.

+PDS and –PDS variants had generally lower morning soil temperatures under surface (50 mm) than +PCT and –PCT during growing period. The way of autumn soil preparation was already important in the depth of 150 mm. In +PDS and +PCT variants the trend was similar as in the depth of 50 mm, however –PDS technology reached higher temperatures than –PCT technology. Afternoon temperatures in the depth of 50 mm ranged unambiguously between about 0.40 and 0.75°C and were higher in de-stoning than in conventional technology. In the temperature sum for growing period between +PDS and +PCT variants it was about 58.4°C and between –PDS and –PCT variants about 39.9°C, in the depth of 150 mm it was again true for +PDS and +PCT variants.

Variants, where de-stoning was applied in, prepared optimum conditions for tuber set and growth due to soil sifting in the whole topsoil horizon. Reduced soil compaction was a positive factor. Soil was more porous with lower bulk density; upper soil layers were better and faster warmed up. On the other hand, characteristics of soil water content were less favourable compared to technologies of conventional spring soil preparation, soil was able to dry out faster and more, however, it did not negatively influence results of tuber yields in conditions of the given experimental years.

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**ABSTRAKT**

**Vliv různých způsobů přípravy půdy před výsadbou na výnosy hlíz brambor a na fyzikální vlastnosti a teplotu půdy**

V letech 1997 až 2000 byl na základě přesných polních pokusů hodnocen vliv technologií podzimního (orba nebo setí vymrzající meziplodiny) a jarního zpracování půdy (konvenční technologie – kypření před sázením, kultivace a ošetření herbicidem po sázení nebo technologie odkameňování – rýhování a separace hrud a kamenů před sázením a ošetření herbicidem po sázení) na výnosy hlíz brambor, hydrofyzikální ukazatele půdy (zjišťované v období vegetace) a teplotu půdy (měřenou denně v 7 a 14 h v hloubkách 50 a 150 mm). Byly nalezeny statisticky významně vyšší výnosy hlíz u technologií s odkameňováním oproti technologiím s konvenční jarní přípravou půdy, a to v průměru o 3,73 $t.ha^{-1}$ (v kombinaci s technologií s orbou) a 9,04 $t.ha^{-1}$ (technologie bez orby), nižší penetrometrický odpor po sázení a před sklizní a s tím související nižší objemová hmotnost půdy (diference v průměru 0,03 $g.cm^{-3}$). Hodnoty pórovitosti byly u obou technologií s odkameňováním statisticky významně vyšší (v průměru všech měření o 1,34 %). Objemová a relativní vlhkost půdy a maximální vodní kapacita byly naopak u technologií s odkameňováním statisticky významně nižší než u konvenčních technologií, a to v průměru o 1,10, resp. 3,45 a 2,83 %. Technologie s odkameňováním měly obecně v průběhu vegetace za následek nižší ranní teploty půdy v hloubce ornice 50 mm, ale odpolední teploty v hloubce 50 mm byly zcela jednoznačně o 0,40 až 0,75 °C vyšší než u konvenčních technologií (v sumě teplot za vegetaci u kombinací s orbou na podzim a s odkameňováním na jaře o 58,4 °C oproti technologii s podzimní orbou a konvenční jarní přípravou půdy a u technologie s vynecháním podzimní orby a odkameňováním na jaře o 39,9 °C, v hloubce 150 mm to platilo jen u kombinací variant s uplatněním orby).

**Klíčová slova:** brambory; příprava půdy; technologie odkameňování; fyzikální charakteristiky půdy; teploty půdy

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# The use of molecular genetic techniques to potato variety identification

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**ABSTRACT**

The RAPD procedure and SSRs analysis were used to study genetic diversity of 30 potato (*Solanum tuberosum*) genotypes, representing mainly Czech potato cultivars. Amplification with six decamer RAPD primers generated 66 DNA fragments, ranging in size from 178 bp to 1847 bp, of which 46 were polymorphic products. Twenty-two primer pairs flanking simple sequence repeats (SSRs) were examined for their potential use in DNA fingerprinting of potato. Total of 76 bands of sizes ranging from 98 to 500 bp were amplified, 56 bands were polymorphic. Similar matrixs derived from fourteen SSRs and six RAPD were able to distinguish selected cultivars. The utilisation of both approaches is discussed.

**Keywords:** potato; *Solanum tuberosum*; DNA polymorphism; RAPD; SSRs; microsatellites; cultivar identification

The identification of varieties of agricultural crops is important at every stage of the agri-production chain. All those involved in crop production – plant breeders, seed producers, merchants, farmers and other users – require benefit from variety identification and discrimination. There is also an increasing interest in the descriptive characterisation in the context of intellectual property protection and verification or quality control.

The contemporary list of registered potato varieties in the Czech Republic contains 102 genotypes including 32 of Czech origin. These varieties are described by morphological characteristics such as flower colour, growth habit, leaf type, disease reaction and sprout and tuber type. The morphological description seems to be insufficient for precise and fast identification of unknown samples and it would be convenient to complete them by means of molecular genetic techniques. Nowadays, there are available a few molecular assay systems which detect polymorphism at DNA level, including probe-based (RFLP), amplification-based (RAPD, SSRs analysis) and second generation methods such as AFLP.

One of these approaches is simple sequence repeats (SSRs) analysis. SSRs are also referred to as short tandem repeats or microsatellites. SSRs or microsatellites are highly polymorphic tandemly repeated DNA sequences with a basic repeating unit of 2–8 that are flanked by conserved DNA sequences. The polymorphism found in microsatellites is due to variations in the copy number of this basic repeat unit. The most frequently observed repeat in plant species is $(AT)_n$ which has been observed to be randomly distributed across the genome, tri- and tetra-nucleotide repeats are also abundant within plant species (Ramel 1997). This class of DNA markers is attractive because of their abundance and relatively simple technical requirements. Simple sequence repeats have demonstrated high levels of polymorphism in many plant species including barley (Saghai Maroof et al. 1994), rice (Yang et al. 1994) and so on. Microsatellites were used to study polymorphism among potato cultivars (Provan et al. 1996b, Schneider and Douches 1997), to study somatic hybrids of potato (Provan et al. 1996a) and in many other applications.

The second method involves the use of random amplified polymorphic DNA – RAPD (Williams et al. 1990). The RAPD marker system is based on the PCR amplification of random DNA with single primer of arbitrary nucleotide sequence (usually 10-mer). Polymorphism is based on the disruption or displacement of homologous target sites between individuals which results in the loss of a product. This method largely generates dominant markers. The variation found out using RAPD is comparatively large without knowledge of sequences, high number of loci is analysed in one sample. These advantages, together with relatively easy technical procedure, are reasons, why RAPD is abundantly used for variety identification and study of genomes of various crops. RAPD was used to study polymorphism among potato cultivars (Demeke et al. 1993), to study interspecific asymetric hybrids of potato (Rasmussen et al. 1997) and in many other applications.

Our study has been aimed at developing useful molecular markers for characterisation of potato varieties by means of RAPD and SSRs analysis.

## MATERIAL AND METHODS

### Plant material and DNA isolation

DNA polymorphism has been assessed in 30 cultivars registered in the Czech Republic:

a) very early variety: Karmela, Koruna, Krasa, Krystala, Velox, Vera, Rosara, Impala

b) early variety: Karin, Kobra, Kreta, Veronika, Vilma, Secura, Dali

Table 1. List of the RAPD ans SSRs primers used in this study

| RAPD | | SSR | | | SSR | | |
|---|---|---|---|---|---|---|---|
| name | primer sequence 5'–3' | name | | primer sequence 5'–3' | name | | primer sequence 5'–3' |
| 308 | AGCGGCTAGG | STM 0040 | F | GCAATAATGGCCAACACTTC | PIP | F | TGTACTGGGGAGCCTCAAAG |
| 131 | GAAACAGCGT | | R | TGGGAAATGTTAGTCAAAAATAGC | | R | AATTTTAACCTCGTGACATGGG |
| 184 | CAAACGGCAC | STM 0051 | F | TACATACATACACACACGCG | STM 0004 | F | CGAGGGCGTAAACTCATGATA |
| SC10-4 | TACCGACACC | | R | CTGCAACTTATAGCCTCCA | | R | AGGTTATTGTGGACACAGTCTTCA |
| P71 | GCATCTACGC | STM 1008 | F | GTACACAGCAAAATAGCAAG | STM 1024 | F | ATACAGGACCTTAATTTCCCCAA |
| P72 | CGGCCACTGT | | R | TAGACACTCTCACATCCACT | | R | TCAAAACCCAATTCAATCAAATC |
| | | STM 1009 | F | ATTAGCATACGACTCAAC | STM 1041 | F | GTTGAGTAGAAGGAGGATT |
| | | | R | TTATTTTCATTTTTCAGC | | R | CCTTTGTCTTCTGCTTTTG |
| | | STM 1020 | F | TTCGTTGCTTACCTACTA | STM 1064 | F | GTTCTTTTGGTGGTTTTCCT |
| | | (II KIG) | R | CCCAAGATTACCACATTC | (Legast) | R | TTATTTCTCTGTTGTTGCTG |
| | | STM 1045 | F | GAAGTTTTATCAGAATCC | STM 1069 | F | ATGCTAACTTGGACACTTA |
| | | | R | ATCACCTCATCAGCAATC | (Lepdsgen) | R | AGTCTCTCAGGAGGATTAC |
| | | STM 1050 | F | GTACATATATACAATTATCTAACCG | STM 1104 | F | TGATTCTCTTGCCTACTGTAATCG |
| | | | R | TTCTCTATGTTAGGCTAGAGTG | | R | CAAAGTGGTGTGAAGCTGTGA |
| | | STM 1056 | F | AGGTAAGTTTTATTTTCAATTGC | STM 2002 | F | AGAACCATTTGATGCATATCCC |
| | | | R | GGGTATGGGAATAGGTAGTTT | | R | TTGTAAGTTTGGGATGAAGCG |
| | | STM 1102 | F | GGAAGAATTTTGTAGGTTCAA | STM 2005 | F | TTTAAGTTCTCAGTTCTGCAGGG |
| | | | R | AAAGTGAAACTTCCTAGCATG | | R | GTCATAACCTTTACCATTGCTGGG |
| | | PP (SSR 7) | F | CAACCAACAAGGTAAATGGTACC | STM 2020 | F | CCTTCCCCTTAAATACAATAACCC |
| | | | R | TGGTCTGGTGCATTAGAAAAAA | | R | CATGGAGAAGTGAAAACGTCTG |
| | | PIG (SSR 2) | F | CTTGCAACTTGTTAGTACCCCC | STM 3012 | F | CAACTCAAACCAGAAGGCAAA |
| | | | R | AAATCCTTTGTGACCTCCCC | | R | GAGAAATGGGCACAAAAAACA |

STM primer sets are listed in Milbourne et al. (1998), primers PP a PIG in Schneider and Douches (1997) and primer PIP in Provan et al. (1996a); 308, 131, 184 (Demeke et al. 1993), SC10-4 (Waugh et al. 1992); P71 and P72 are random chosen sequences

c) medium-early variety: Keřkovské rohlíčky, Korela, Krista, Tara, Satina, Vladan, Granola, Folva
d) medium-late variety: Amylex, Javor, Ornella, Pacov, Zlata, Saturna, Asterix

DNA was extracted from fresh leaf material collected in greenhouse and in field, from *in vitro* plants and from tubers using Dneasy Plant Mini kit (Qiagen). The DNA samples were quantified by fluorometry (Perkin Elmer) and were diluted to 200 ng/µl for PCR.

### RAPD analysis

PCR for RAPD analysis was carried out using six decamer oligonucleotide primers (Table 1). Amplification reactions were performed in volumes of 25 µl consisting 200 ng template DNA, 200 µM each of dATP, dCTP, dGTP and dTTP (Sigma), 3.2 µM primer (IDT – Integrated DNA Technologies, Inc.), 1× DyNAzyme™ II buffer (10 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100), 0.7 U DyNAzyme™ II polymerase (Finnzymes). RAPD reaction was performed in PTC 100 Termo Cycler. Amplification conditions were as follows: one cycle 94°C – 3 min, forty-one cycles 94°C – 1 min, 37.5°C – 1 min 40 sec, 72°C – 2 min, one cycle 72°C – 10 min. Amplification products were analysed on 1.2% agarose gel and visualised by staining with ethidium bromide (0.25 µg/µl).

### SSRs analysis

Simple sequence repeat polymorphism analysis was performed using the 22 primer sets (Table 1). DNA from each potato sample was amplified in a 25 µl reaction consisting of 200 ng template DNA, 200 µM each of dATP, dTTP, dCTP and dGTP (Sigma), 10 pmol each flanking primer (IDT – Integrated DNA Technologies, Inc.), 1× reaction buffer – magnesium free (50 mM KCl, 10 mM Tris-HCl, pH 9.0 at 25°C, 0.1% Triton X-100) and 0.5 U Taq DNA polymerase (Promega). The $MgCl_2$ concentration and the annealing temperature for this reaction were optimised for each primer set. Amplification was performed using the thermal cycler PTC-100, and the reaction conditions were described in Provan et al. (1996b). The PCR products were separated on a 3% agarose gel stained with ethidium bromide (0.25 µg/ml).

### Statistical analysis

The products of RAPD and SSRs analysis were visually scored. Band presence was indicated by 1 and absence by 0. The similarities of profiles were estimated with the Jaccard's coefficient (Jaccard 1908). The resulting data was processed with cluster analysis using the UPGMA (unweighted pair group average). Data analysis was performed using software SPSS Base+.

## RESULTS AND DISCUSSION

### Variation detect by RAPD fingerprintings

Polymorphism was observed among 30 potato cultivars using all RAPD primers (Table 1). Amplification with six decamer primers generated 66 DNA fragments, ranging in the size from 178 bp to 1847 bp, of which 46 were polymorphic products. Primers SC10-4 and P72 produced the most polymorphisms, each enables the distinction among 15 of 30 cultivars. Together the RAPDs of primers SC10-4 and P72 distinguished 28 of the 30 potato varieties. The polymorphisms produced by primer 308 distinguished 10 cultivars and the use of primer 131 made the identification of 8 varieties possible. The ability of a few randomly selected primers to produce RAPDs capable of differentiating all 30 examined cultivars (Figure 1). This fact demonstrates the potential of this method for distinguishing and identifying potato cultivars.

As for the precision and the reproducibility of the results, in most cases, the most prominent amplified DNA products were readily reproducible. However, a few primers showed certain polymorphism (namely at the products of extreme sizes – below 250 bp and above 1500 bp). This polymorphism proved as disappearing of some bands or as changes of band spectrum (it means, the same products were amplified but with different intensity, the result was the alternation of the appearance of

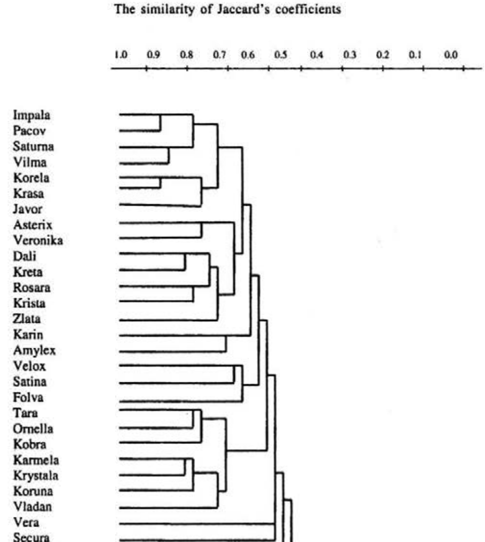


Figure 1. Distinction of potato cultivars using RAPD primers; dendrogram of 30 selected potato cultivars constructed using UPGMA (unweighted pair-group method with arithmetical averages) based on similarity matrix of Jaccard's coefficients; dendrogram shows the similarity of RAPD's profile of selected genotypes, which were produced by using all six primers

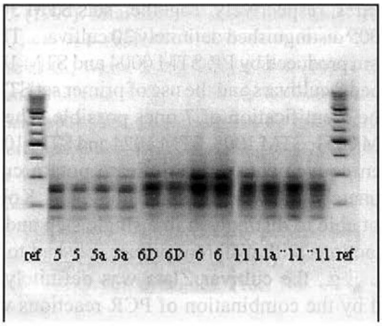


Figure 2. Test of reproducibility of RAPD reaction (primer 308); ref – weight marker (250 bp ladder); potato cultivars: 5 – Impala, 6 – Keřkovské rohlíčky, 11 – Saturna; 5, 5a – two freshly isolated samples; 6D, 6 – freshly DNA and older sample; 11, "11 – two independent prepared RAPD reaction

gels). This variability was especially observed at primer 131. A lot of authors discussed about this problem in their studies (Demeke et al. 1993, Hallden et al. 1996). The reason for this is not clear, but it may occur from changes in the annealing sites. Less efficiently amplified secondary or tertiary products could result from weaker, mismatched annealing of the primer to one or more target sites which define a product (Demeke et al. 1993).

However, if a care is taken and procedures standardised, it is possible to maximise reproducibility with most primers. One part of our efforts was focused on the problem of RAPD reproducibility. Experiments were carried out with seven selected cultivars (Impala, Keřkovské rohlíčky, Saturna, Asterix, Granola, Vilma, Kreta) and four RAPD primers (P72, SC10-4, 308, 131) which revealed the most polymorphism in previous assays. As templates in PCR were used two fresh DNA samples (two individuals of one cultivar) and DNA samples from older reserves. Any difference in RAPD products was not found among distinct DNA samples of the same cultivar. It was also tested that products of two independently prepared reactions are identical (Figure 2). These results indicate that it is possible to reach the sufficient reproducibility of RAPD.

**Variation detect by microsatellite fingerprintings**

The level of variation detected with SSRs probes was maximised in this study by preselecting primers that would detect the highest levels of polymorphism. For this purpose, twenty-two primer sets were screened against a small test series, consisting of five cultivars – Vera, Impala, Dali, Karmela, Vladan. Twenty-two primer sets amplified 76 fragments of sizes ranging from 98 to 500 bp (Table 2). Altogether, 56 polymorphic bands were amplified. Based on this information we could define the most suitable primer sets for the discrimination and the identification of potato varieties. We chose the scale from 1 (convenient) to 5 (inconvenient) to evaluate the used primer pairs (not shown). Of twenty-two sets of primers, fourteen were convenient for the cultivar identification (level 1, 2, 2–3). These primer pairs produced highly reproductive bands which were characterised by good quality and considerable polymorphism. Similar results were reached for primer pairs tested by Schneider and Douches (1997). On the other hand, the products of primer sets STM 2002, STM 1104, STM 1041, STM 1045 and STM 1056 were monomorphic.

This study demonstrated that variability exists for seventeen of twenty-two SSRs tested within potato cultivars. On average, 2.5 band variants were observed per amplified SSR. A similar level of variation was observed by Veilleux et al. (1995) using DNA from anther-derived monoploids and diploids of potato or by Schneider and Douches (1997) in their study of polymorphism of North American potato cultivars. This level of polymorphism is substantially bellow that observed for other crop species. For example, nine alleles per locus were identified in rice (Yang et al. 1994) and for barley, 17.7 alleles per locus were observed by Saghai Maroof et al. (1994). The disparity on the level of SSR variation found within heterozygous potato cultivars and highly inbred self-pollinated crops is not fully explained, although a lot of loci are examined in potato and SSR variation is studied in more crop species. However, if SSR variation is a result of mismatch and unequal crossing-over, the reduced number of meiotic events in the breeding and maintenance of a vegetatively propagated crop may explain the lower allelic diversity compared to highly inbred, seed propagated crops (Schneider and Douches 1997).

Table 2. Summary of the number of each type of assay performed, the total number of products obtained per assay type, the number which were polymorphic, the mean number of products per assay (primer or primer pair) and the overall percentage of polymorpic products

| | Total number of assays | Bands scored | | Mean number of bands per assay | % polymorphic bands |
|---|---|---|---|---|---|
| | | total | polymorphic | | |
| RAPD | 6 | 66 | 46 | 11 | 69.7 |
| SSR | 22 | 76 | 56 | 3.5 | 73.7 |

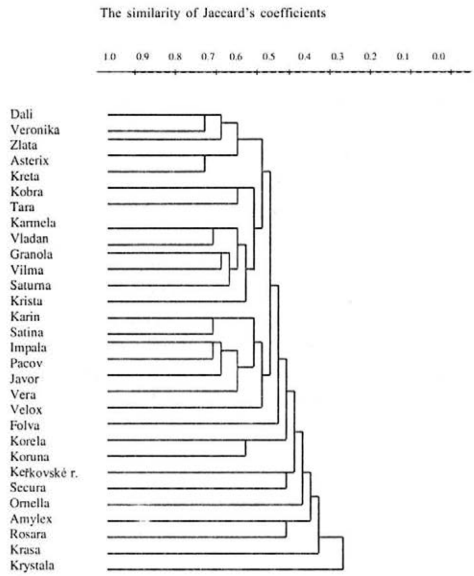


Figure 3. Distinction of potato cultivars using 14 SSRs primers; dendogram of 30 selected potato cultivars constructed using UPGMA (unweighted pair-group method with arithmetical averages) based on similarity matrix of Jaccard's coefficients; dendrogram shows the similarity of SSRs profile of selected genotypes, which were produced by the 14 most suitable primer sets

Polymorphism was observed among 30 selected cultivars using any of 14 primer sets (Figures 3 and 4). The primer sets STM 3012 and STM 2005 produced the most polymorphism and we were able to differentiate 12 and 10 varieties, respectively. Together, sets STM 3012 and STM 2005 distinguished definitely 20 cultivars. The polymorphism produced by PP, STM 0004 and STM 1020 distinguished 8 cultivars and the use of primer set STM 1064 made the identification of 7 ones possible. The primer sets STM 0051, STM 1008, STM 1024 and STM 1069 have not been convenient to discriminate potato cultivars. Unfortunately, a few varieties (Dali, Zlata, Kobra) we were not able to distinguish in a single step and further PCR reactions with different primer sets had to be performed. E.g., the cultivar Zlata was definitely distinguished by the combination of PCR reactions with the sets PIP and PP. For practical purposes, this procedure is relatively laborious and time consuming.

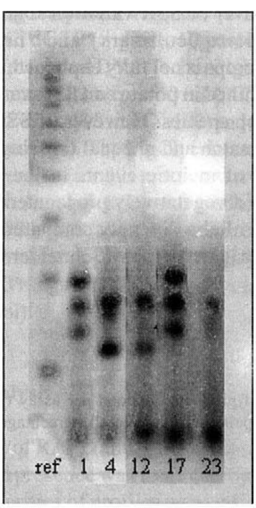


Figure 4. Amplification of genomic DNA from 5 potato genotypes using STM 1102; ref – weight marker (100 bp ladder); potato cultivars: 1 – Vera, 4 – Rosara, 12 – Asterix, 17 – Vilma, 23 – Secura

The basic criteria for a molecular marker preferred for cultivar identification listed Bailey (1983). These include distinguishable inter-cultivar variation, minimal intra-cultivar variation, environmental stability and experimental reproducibility. Both methods, RAPD and SSRs analysis, have the potential to satisfy all these criteria. Our study has shown that variation among potato cultivars can be revealed using both approaches. But our preliminary results also proved that SSRs did not generate unique fingerprints for all examined cultivars in one step and further evaluation would be necessary. So far acquired results indicate that RAPD is more suitable for rapid identification of potato.

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## ABSTRAKT

### Identifikace odrůd brambor molekulárně genetickými technikami

Genetická diverzita 30 genotypů bramboru (*Solanum tuberosum*), reprezentující převážně české odrůdy, byla hodnocena pomocí techniky RAPD (Random amplified polymorphic DNA) a analýzy SSRs (mikrosatelitů). Výsledkem RAPD reakce se šesti dekamerickými primery bylo 66 fragmentů DNA, z toho 46 bylo polymorfních. Velikost amplifikačních produktů se pohybovala v rozmezí od 178 do 1847 bp. Analýza mikrosatelitů byla prováděna s 22 páry primerů, výsledkem bylo 76 produktů, z toho 56 polymorfních. Velikost fragmentů byla v rozmezí od 98 do 500 bp. Souhrnné výsledky amplifikace obou metod jsou uvedeny v tab. 2. K hodnocení podobnosti RAPD profilů a produktů analýzy mikrosatelitů byl použit Jaccardův koeficient, takto získaná data byla dále podrobena klastrové analýze (UPGMA). Naše výsledky získané pomocí RAPD analýzy naznačují, že jde o rychlou techniku, která by mohla být využívána při identifikaci genotypů brambor. Sada náhodně vybraných RAPD primerů je schopna rozlišit vybrané odrůdy, z čehož vyplývá značný potenciál metody pro identifikaci odrůd bramboru (obr. 1). U analýzy mikrosatelitů byla nejprve provedena preselekce, během níž bylo stanoveno 14 párů primerů vhodných ke studiu polymorfismu vybraných odrůd. Dendogram (obr. 3) sestavený na základě podobnostní matice ukazuje, že 14 vybraných sad může rozlišit zvolené odrůdy. Některé odrůdy (Dali, Zlata, Kobra) nebylo možné jednoznačně identifikovat použitím jedné sady primerů, ale bylo nutné provést další PCR reakce s různými sadami primerů. Odrůda Zlata byla např. jednoznačně určena kombinací PCR reakcí s primery PIP a PP. Takovýto postup je však zdlouhavý, poměrně pracný a pro potřeby rychlého posouzení nevhodný. Při studiu polymorfismu vybraných odrůd brambor se ukázalo, že mikrosatelity jsou cenné genetické markery, které se vyznačují dostatečnou variabilitou a vysokou reprodukovatelností. Je diskutována možnost využití obou metod.

**Klíčová slova:** brambory; *Solanum tuberosum*; polymorfismus DNA; RAPD; SSRs; mikrosatelity; identifikace odrůd

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# Yield reduction and losses due to tuber infection caused by potato late blight

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**ABSTRACT**

In exact field trials conducted during 1997–2000 yield losses and losses due to tuber infection caused by late blight were observed in Lukava and Dali potato varieties. Sets of untreated variants, variants treated with different fungicides used for the whole season, various fungicide programs and ecological treatments (copper preparations, max. 3 kg $Cu.ha^{-1}$) were investigated. 5–7 treatments were applied in fungicide variants, in case of ecological treatments 2–4 sprays were applied per season. Occurrence of foliage disease was very strong in experimental years; conditions for tuber infection were only suitable in 2000. Total haulm destruction of untreated crop occurred in 27–38 days from the first blight symptoms, tuber yield reached 19.04–35.4 $t.ha^{-1}$. Individually applied fungicides increased yield about 2–115%, fungicide programs about 13–106% and ecological treatments about 0–18% compared to untreated variant. Tuber infection was 0–1.2% in all trial variants in 1997–1998, tubers were not infected by late blight in 1999. 35.7% of tubers were infected in untreated variant, 7.44–54.74% of tubers in fungicide variants and 7.69–37.3% of tubers in fungicide programs in 2000. 30–54.74% of tubers were infected in ecological treatments. Infected tubers were distributed in soil prior to harvest that year and they significantly reduced final yield. Losses caused by late blight are very serious, but they could not be predicted and generalized for respective year. They are set by weather conditions, which affect disease occurrence, characteristics of used variety and protection system. Trial results proved economic importance of late blight and decisive effect of chemical protection on reducing yield and qualitative losses.

**Keywords:** potatoes; late blight; yield losses; tuber infection

Late blight, which is caused by facultative biotrofical parasite *Phytophthora infestans* (Mont.) de Bary, is the most serious potato disease all over the world. Except for marginal arid areas, where potatoes must be grown under irrigation and late blight has not suitable conditions there, potato growing without disease control is very risky and less effective. Under sub-tropic and tropic conditions of developing countries potatoes gradually become basic food; it is verified by continuous increase in potato areas. Economic terms and lack of knowledge often do not allow intensive control, potato late blight is a limiting factor of yields in those localities. Countries of well-developed potato growing pay an extraordinary attention to the control and costs represent 10–15% share of total costs of potato growing. Application of fungicides is still a decisive part of control. A number of treatments differs according to conditions in respective season, grown varieties and utility types, according to the control intensity, used preparations and local traditions and customs. Most often number of sprays ranged between 4 and 10–15 per season. From above mentioned it is apparent that control is very expensive and demanding for work arrangement. Sprays must be applied on the right term, particularly before epidemics and in the period of high infection pressure. It is often complicated in intensive rainfalls, when it is not possible to go into crops. Protection of tubers against late blight is difficult; it is solved by some cultural practices and vegetation ending on a suitable term.

Current population of late blight in the whole world is characterized by occurrence of new strains with higher pathogeneity and ability to infect plants in wider range of ecological conditions. Observed distribution of sexually rising oospores is a potential of development of recombinant strains with new characteristics and oospore resistance to external effects allow surviving in soil. All those aspects increase requirements for fungicide protection, which is necessary. Disease control is very difficult in organic agriculture managements. Suitable bioagent allowing practical using have not been found in the biological control yet.

Great prospective contributed to the development of resistant varieties. However, difficult and a long-term procedure and significant success in using of all hitherto known methods are expected in time horizon of 10–15 years. Wide applying resistant varieties are first expected in sub-tropic and tropic localities of developing world, where lower requirements are on quality assortment and resistance to late blight is preferred.

Losses caused by late blight involve yield reduction due to leaf area destruction and losses due to tuber infection. Yield reduction can range in wide spectrum according to infection pressure of disease, used variety and locality. Crop developmental stage in period of epidemics has an essential effect. Despite intensive fungicide control, losses are estimated in range of 5–20%. For example in Ireland, there are estimated annual mean losses of 8% (Copeland et al. 1992). In central and eastern Eu-

Table 1. Active ingredients used in trial variants

| Set of variants | Number of variants in set | Active ingredients in used fungicides | Number of sprays per season |
|---|---|---|---|
| Solely applied fungicides | 15–18 | dimetomorph + mancozeb, fluazinam, chlorothalonil, fentinhydroxid, oxychlorid Cu, cymoxanil + mancozeb, mancozeb, folpet, benalaxyl + mancozeb, metalaxyl + mancozeb, metiram, metalaxyl + oxychlorid Cu, propamocarb HCl + mancozeb, oxydixyl + mancozeb, cymoxanil + oxadixyl + mancozeb | 5–7 |
| Fungicide programs | 18–20 | dimetomorph + mancozeb, fluazinam, chlorothalonil, fentinhydroxid, cymoxanil + mancozeb, mancozeb, benalaxyl + mancozeb, metalaxyl + mancozeb, metalaxyl + oxychlorid Cu, propamocarb HCl + mancozeb, cymoxanil + oxadixyl + mancozeb | 5–7 |
| Ecological treatment | 2–4 | oxychlorid Cu | 2–4 |

rope, where control is generally on lower level, mean yields range between 7.6–20.3 t.ha$^{-1}$ and potato late blight is a main cause of such low yields. For example, only 12–40% of areas was treated with fungicides in Poland during 1993–1997 and average number of sprays was lower than 2. It results in yield losses of 22–40% (Guzowska 1999). In the Czech Republic, yield losses are estimated on average of 10% and losses due to tuber infection of 5% (Hausvater 1999) on current level of control (at important growers using 4–10 treatments).

Direct losses due to late blight are difficult to be determined. More often effect of yield growth is indicated in per cent in treated crops compared to control without protection against late blight. In trials of Great Britain, e.g. effect of fungicide control on various levels in 38 field trials was exhibited by yield increase from 0 to 118.5%, on average about 32.2%, it corresponds to 12.93 t.ha$^{-1}$ (Hims et al. 1995).

The solution aim of the given problems was obtaining knowledge of yield losses and losses due to tuber infection caused by late blight, and/or comparison of untreated control and different variants of fungicide protection.

## MATERIAL AND METHODS

In exact field trials losses due to late blight were observed in yields and tuber infection. Resource of loss evaluation was comparison of different variants of chemical protection with an untreated control. Trials were established in central potato-growing area at Valečov Research Station during 1997–2000. Common cultural practices with herbicide application were used for the trials. Trials were established in 4 replications with size of individual plot 3 × 7.5 m, 100 plants were planted per plot. Investigation was done in medium-late variety Lukava susceptible to tuber and foliage late blight in the first three experimental years. After excluding from potato assortment it had to be placed with early variety Dali, which is also susceptible to tuber and foliage late blight. Yield and tuber infection were determined in a variant without late blight control, in variants with different fungicides applied for the whole season (15–18) or with different fungicide programs (18–20) and in ecological treatment (2–4), where maximum 3 kg Cu.ha$^{-1}$ for season could be applied (Table 1).

Registered preparations were used in 5–7 applications of common dosage in treated variants; total copper dose was split in 2–4 applications in ecological treatment.

Treatment was always started according to negative late blight forecast (Ulrich and Schrödter 1966). Meteorological parameters were observed directly on plot with automated meteorological station. Further sprays followed in 1–2 weeks interval according to weather and disease infection pressure.

Tuber infection was measured 4–6 weeks after harvest; in 2000 it was also measured before harvest during growing period with respect to very suitable conditions for tuber infection and fast tuber decay in soil.

Results were evaluated with variance analysis and Duncan test.

## RESULTS AND DISCUSSION

Results were obtained in the years, when conditions of occurrence of foliage disease were very favourable, except for year 2000, but less suitable for tuber infection. Figure 1 presents progress of infection on untreated foliage in experimental years.

In 1997 first infection by late blight was recorded in the trials on July 18, complete haulm destruction in untreated variant occurred in 38 days. Tuber yield reached 35.4 t.ha$^{-1}$. Yield increase about 11–62% was reached in variants, where chemical control was applied using individual fungicides during the whole growing period. Applying fungicide programs, when 2–4 fungicides were used in total number of 6 treatments, yields increased about 38–64%. In case of ecological treatment using 2–4 copper applications on different dates, yield increase about 3–18% was achieved compared to untreated variants. With regard to unfavourable conditions for tuber infection in August, tuber infection was very low (0–1.2%) and differences were not significant.

In 1998, onset of disease was very early and the first infection was determined on July 3. Complete haulm de-

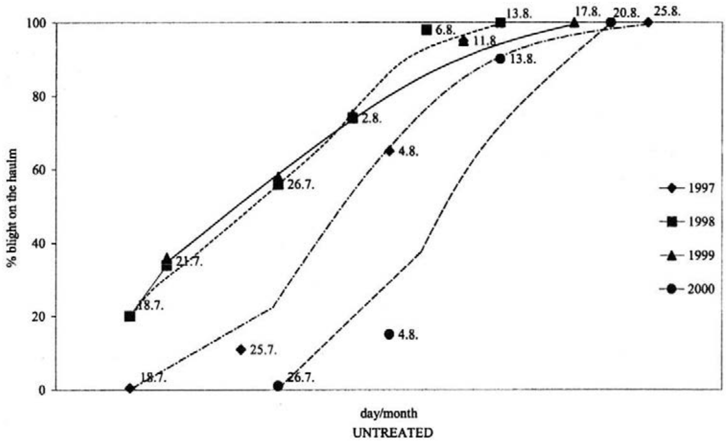


Figure 1. Blight progress 1997–2000

struction of untreated crop occurred in 41 days. Tuber yield reached 19.97 t.ha$^{-1}$. Individually applied fungicides increased the yield about 18–100%, fungicide programs about 36–102% and ecological treatments about 0–14%. There was again minimum tuber infection from 0.0 to 0.79% with non-significant differences among variants that year.

Year 1999 was characterized by high infection pressure of disease and crop infection from July 12, and haulm destruction in 34 days, when untreated with fungicides. Tuber yield without crop treatment reached 23.48 t.ha$^{-1}$. Individual fungicides increased yield about 22–66%, fungicide programs about 32–56% and ecological treatments of copper about 1–21%. Lack of intensive rainfall in August and beginning September, which are a condition of tuber infection caused that tubers were not infected by late blight in the trial that year.

In 2000 infection pressure of late blight was also high, but infection onset was delayed. First disease occurrence was found in trials on July 25. Total haulm destruction occurred in 27 days, i.e. the fastest over 4 experimental years. Found tuber yield in untreated variant was 19.04 t.ha$^{-1}$. Fungicides increased yield about 2–115%, fungicide programs about 13–106% and ecological treatment about 3–13%. Tuber infection was extraordinary high, active source of infection in foliage, intensive rainfall in the first 10 days of August and suitable temperatures contributed to this fact. Moist soil and optimum temperatures promoted extraordinary fast decay of tubers in soil before harvest. 35.7% of tubers was infected in untreated control on August 30. 7.44–54.74% of tubers was infected in variants with individual fungicides, 7.69–37.3% in fungicide programs and 30.0–54.74% of tubers in ecological treatments (Figure 2). Differences in infection were non-significant to highly significant according to applied fungicide or fungicide program and non-significant in ecological treatments compared to untreated variant.

Contradistinction to years 1997–1999, yields of the year 2000 were markedly reduced by decrease in infected tubers, which were distributed in soil, those results express total losses, i.e. losses caused by leaf area reduction and losses due to tuber infection.

Tuber yields are present in Figures 3–5, tuber infection in individual years in Figure 6.

From results obtained, there is apparent that potato late blight is a disease, which affects yields and tuber quality in a decisive way. Difference in yields between fungicide-treated crop and untreated crop are tenths of per cent in conventional technology of growing. Years with low occurrence of foliage disease are an exception. Leaf area infection and yield reduction does not always mean tu-

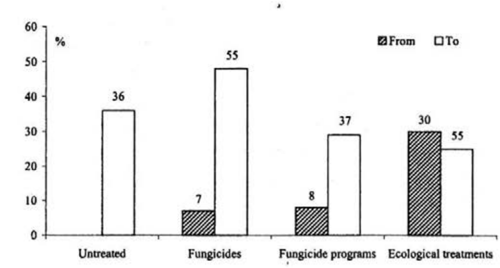


Figure 2. Tuber blight (%), 2000

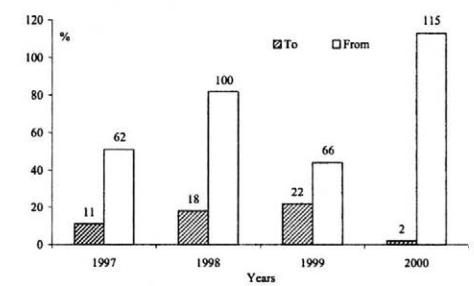


Figure 3. Increase in the yields (%), fungicides (5–7 treatments)

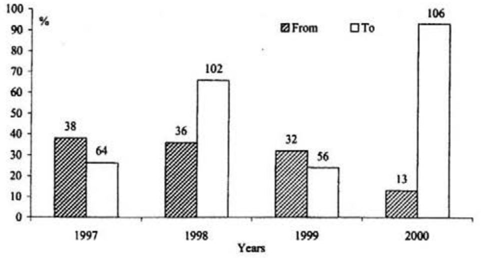


Figure 4. Increase in the yields (%), fungicide programs (5–7 treatments)

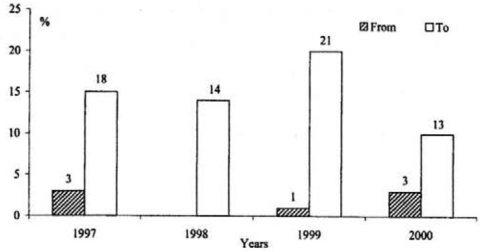


Figure 5. Increase in the yields (%), ecological treatments (2–4 sprays, 3 kg Cu per season)

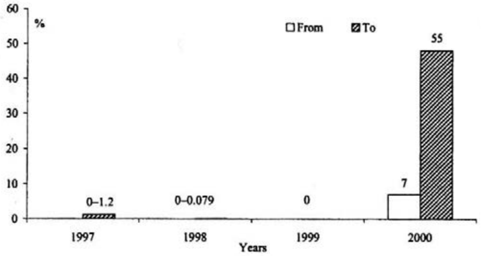


Figure 6. Tuber blight (%), all trial variants

ber infection. Late blight control is a necessary, but very effective measure for successful growing of this crop. Trials also showed that effect of fungicide protection ranges in wide spectrum and its efficiency depends on applied fungicide and fungicide program. It is also apparent that losses could not be predicted and generalized

Disease occurrence in crop is particularly determined by weather conditions in respective year and locality and used variety, its susceptibility and earliness. A lot of other factors are added to this fact, which could influence losses such as planting date and plant emergence, crop structure, nutrition, cultural practices and protection system.

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**ABSTRAKT**

**Redukce výnosů a ztráty napadením hlíz způsobené plísní bramborovou**

V přesných polních pokusech v letech 1997–2000 byly sledovány ztráty ve výnosech a napadení hlíz způsobené plísní bramborovou u odrůd Lukava a Dali. Byly pozorovány soubory variant bez ošetření, s ošetřením různými fungicidy použitými po celou sezonu, rozdílnými fungicidními programy a ekologickým ošetřením (měďnaté přípravky, max. 3 kg $Cu.ha^{-1}$). U fungicidních variant bylo aplikováno 5–7 ošetření za sezonu, v případě ekologických ošetření 2–4 postřiky. Výskyt choroby v nati byl v pokusných letech velmi silný, podmínky pro infekci hlíz byly vhodné pouze v roce 2000. K úplnému zničení natě u neošetřeného porostu došlo za 27–38 dní od prvních příznaků infekce, výnos hlíz dosáhl 19,04–35,4 $t.ha^{-1}$. Fungicidy aplikované samostatně zvýšily výnos o 2–115 %, fungicidní programy o 13–106 % a ekologická ošetření o 0–18 % v porovnání s neošetřenou variantou. Napadení hlíz u všech pokusných variant v letech 1997–1998 činilo 0–1,2 %, v roce 1999 nebyly hlízy plísní napadeny. V roce 2000 bylo napadeno 35,7 % hlíz v neošetřené variantě, 7,44–54,74 % hlíz u fungicidů a 7,69–37,3 % hlíz u fungicidních programů. U ekologických ošetření bylo infikováno 30–54,74 % hlíz. Napadené hlízy v tomto roce byly zcela rozloženy v půdě před sklizní a významně redukovaly celkový výnos. Ztráty způsobené plísní bramborovou jsou velmi závažné, ale nelze je pro daný rok předpovědět a zobecnit. Jsou dány průběhem povětrnostních podmínek, které ovlivňují výskyt choroby, vlastnostmi pěstované odrůdy a systémem ochrany. Výsledky pokusů prokázaly hospodářský význam plísně bramborové a rozhodující vliv chemické ochrany na snížení výnosových a kvalitativních ztrát.

**Klíčová slova:** brambory; plíseň bramborová; výnosové ztráty; napadení hlíz

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# Changes of selected secondary metabolites in potatoes and buckwheat caused by UV, gamma- and microwave irradiation

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**ABSTRACT**

Changes of total polyphenols, phenolcarboxylic acids and ascorbic acid in potato tubers cv. Kordoba and Rosella and three buckwheat samples (seeds, seedlings and plants: *F. esculentum* Moench, cv. Pyra and Emka and tartarian buckwheat *F. tataricum* Gärtner) induced by UV-C irradiation ($\lambda$ = 253.7 nm, P = 75 W, 0 – control, 42 and 84 h), $\gamma$-irradiation ($^{60}Co$ Artim Prague at doses 0 Gy – control, 50 Gy and 100 Gy) and microwave irradiation (P = 90 W, 0 – control, 200 and 400 s) were investigated. Potato tubers contained in av. 300–680 mg ascorbic acid.kg$^{-1}$ dry matter. By UV-C and $\gamma$-irradiation effect there was a decrease of ascorbic acid content in dry matter of potato tubers observed, by the influence of microwave irradiation an increase by 78% was determined. Total polyphenol content in potato tuber dry matter was 3300–6200 mg.kg$^{-1}$. Potato tuber phenolics were identified by HPLC method with UV detection. The most contained compounds were phenolic amino acid L-tyrosine (2.25 mg.kg$^{-1}$ d.m.), chlorogenic acid (0.05 mg.kg$^{-1}$ d.m.), caffeic acid (0.007 mg.kg$^{-1}$ d.m.), and ferulic acid (0.004 mg.kg$^{-1}$ d.m.). Microwave irradiation caused a decrease of all investigated acids. $\gamma$-Irradiation enhanced L-tyrosine content (by 35–70%), but in other phenolic acids was found their decrease. UV-C irradiation induced an increase of L-tyrosine in cv. Kordoba and a decrease in cv. Rosella. The content of other phenolcarboxylic acids was enhanced by UV-C irradiation. *F. tataricum* Gärtner contained much higher total polyphenol and rutin levels in comparison with *F. esculentum* Moench. With developing of plants total polyphenol and rutin contents increased. UV-C irradiation affected seeds causing an increase of total polyphenols and rutin, $\gamma$-irradiation at lesser doses caused an increase of total polyphenols, at higher doses a contrary effect was determined. Similar effect was observed for rutin. Microwave irradiation did not affect total polyphenols, but a rutin increase was observed with the exception of cv. Emka. In seedlings and plants $\gamma$-irradiation caused a decrease of total polyphenols and rutin. Lesser doses of $\gamma$-irradiation caused the increase of rutin, but at higher doses rutin content decreased. UV-A irradiation in plants enhanced levels of total polyphenols and rutin. In contrary, microwave irradiation caused a decrease of total polyphenols and rutin.

**Keywords:** potatoes; buckwheat; polyphenols; ascorbic acid; phenolcarboxylic acids; rutin; UV-C and UV-A irradiation; $\gamma$-irradiation; microwave irradiation

Polyphenols and ascorbic acid are natural compounds that as secondary metabolites are present in every higher plant and in every its organ. Polyphenols are especially contained in outer layers of the tubers and seeds where they protect an embryo against ultraviolet irradiation (Mazza et al. 2000) regarding the fact that they have an ability to absorb it (Lachman et al. 1998a). Both, polyphenols and ascorbic acid are efficient antioxidants (Lachman 2000 a, b) favourable to human health.

Because the thinning of the stratospheric ozone layer is permitting more UV-B to enter the biosphere, the mechanisms of action of UV-B radiation on plants are of particular current interest (Balakumar et al. 1997). It was determined in *Arabidopsis thaliana* wild-type *Landsberg erecta* (Ler) and the UV-B-sensitive mutant fahl (deficient in UV-absorbing esters of sinapic acid) that UV-B exposure (at doses of 6–7 kJ.m$^{-2}$.d$^{-1}$) decreased dry matter production (Ormrod et al. 1999).

Liu and Mc Clure (1995) investigated barley polyphenol levels in plants that were grown with UV-B (280–320 nm) at levels simulating 25% or 5% ozone depletion on the date of the summer solstice at 40° N latitude, with UV-A (320–400 nm), or with no supplemental irradiation. UV-B increased flavonoid (saponarin and lutonarin) accumulation in both the lower epidermis and the mesophyll; about 40% of the saponarin and 20% of the lutonarin were in the lower epidermis under all experimental conditions. Levels of vacuolar ferulic acid esters were significantly higher in UV-B grown plants on days 10 and 15 and this fraction significantly increased in the lower epidermis. UV-A had no significant effects on growth, photosynthesis or ferulic acid, but it slightly increased flavonoid accumulation. Balakumar and Selvakumar (1998) investigated the total phenol content and the polyphenol oxidase activity in the leaves of cowpea and *Crotalaria* seedlings treated with various UV-B irradiance levels. In both the plants tested, the total phenol content has increased proportionately with the dose of UV-B radiation. However, the concentration of total phenols in the leaves of cowpea was higher under UV-B irradiation compared to *Crotalaria* seedlings. The activity of polyphenol oxidase, on the contrary, has shown reduction in both cowpea and *Crotalaria* seedlings. Effects of solar and UV-B radiation on wheat seedlings (Häder

1996), sensitivity to UV-B of plants growing in different altitudes (Rau and Hofmann 1996), diurnal variation in UV-protective flavonoids (Veit et al. 1995) as well as the protective role of plant pigments in *Picea abies* (L.) Karst. is now intensively investigated.

Pendharkar and Nair (1995) studied phenylpropanoid metabolism in γ-irradiated potato tubers by examination of the pattern of incorporation of radioactivity from U-$^{14}$C-phenylalanine into caffeic acid, chlorogenic acid and the coniferyl and sinapyl moieties of lignin. During a post-irradiation period of 21 days a depletion in chlorogenic acid was observed. This is a result of its impaired synthesis as well as an accelerated conversion of chlorogenic acid to ferulic and sinapic acids and their deposition in lignin. Thomas (1981) also referred the changes in potato phenolic and alkaloidal compounds in irradiated potatoes. γ-Radiation insures the possibility of extending the storage life of different vegetables and fruits, e.g. onions – 0.10 kGy (Khan et al. 1999), garlic (Manniti 1979). Al-Safadi and Simon (1996) have found that γ-radiation accelerated germination of carrot seed in the $M_1$ generation at low doses (0.5 and 1 krad, i.e. 5.0 and 10.0 Gy), whereas higher doses delayed germination. Plant size and root weights were by 20–35% greater than control plants after seeds. Higher doses reduced $M_1$ plant size by > 50% in germinating seed and tissue culture treatments but less for the dry seed treatment. Irradiation of germinating seed and tissue cultures yielded more $M_2$ variation than irradiation of dry seed. Massive dosage of γ-irradiation (1–3 kGy) has been found to induce some particular responses in *Arabidopsis thaliana* (Nagata et al. 1997, 1999). The accumulation of anthocyanin in the aerial parts and the formation of new trichomes were determined. The plants stopped their development, too. Ramamurthy et al. (1992) determined the content of phenolic acids formed during wound healing of γ-irradiated and nonirradiated potato tubers by HPLC. Chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid were detected in small quantities in potato tubers. During wound healing their content increased many fold, and in addition, the neo and crypto isomers of chlorogenic acid accumulated in the wound healing tissue, but tubers irradiated to 100 Gy for sprout inhibition showed significantly lower levels of chlorogenic acid and its isomers. Orsák et al. (2000) investigated in two pea cultivars (Lantra, Menhir) and barley cultivars (Krona, Kompact) changes in total polyphenol content (TP) and major phenolic acids caused by UV-A and γ-irradiation. The changes were determined in seeds, seedlings and plants. In barley cultivars the content of catechol, resorcinol and phloroglucinol type compounds (CRP) was also estimated. UV-A irradiation caused in investigated plants an increase of TP content, γ-irradiation enhanced TP content in barley but in pea it decreased. UV-A and γ-irradiation decreased CRP content in barley. Statistically significant dependence was found between CRP content and the doses of irradiation and the vegetation phase of plants. Cultivars responded differentially to various doses of irradiation in both types of irradiation. The most represented phenolic acids in pea and barley were 2,3-dihydroxybenzoic, sinapic, m-hydroxybenzoic, veratric and vanillic acid. UV-A irradiation caused the apparent increase of these acids both in barley and pea, whereas γ-irradiation caused only little changes in the content of these compounds.

In our study on the irradiation effect of different types of irradiation on the content of polyphenols and ascorbic acid as antioxidants in potato tubers and buckwheat seeds, seedlings and plants subjected to UV-C, γ- and microwave irradiation, it was of interest to know how these types of irradiation influence their content and composition.

## MATERIAL AND METHODS

Samples of buckwheat (*Fagopyrum esculentum* Moench) obtained from Research Institute of Crop Production from Prague-Ruzyně cv. Pyra (diploid) and cv. Emka (tetraploid), tartarian buckwheat (*Fagopyrum ta-*

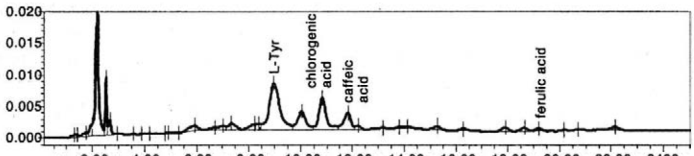


Figure 1. Chromatogram of the HPLC determination of phenolic acids in potato tubers

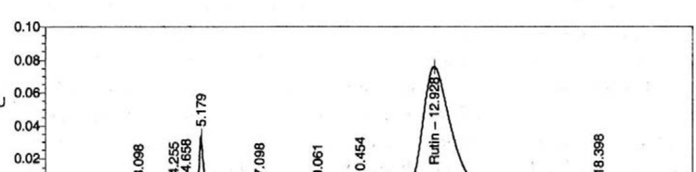


Figure 2. Chromatogram of the HPLC determination of rutin in buckwheat seeds

*taricum* Gärtner) and potato tubers (*Solanum tuberosum* L.) cv. Kordoba and cv. Rosella obtained from the trial field of the Czech University of Agriculture in Prague were subjected to UV-C irradiation (low-pressure mercury vapour lamp, $\lambda = 253.7$ nm, P = 75 W) for 0 (control), 42 and 84 hours (in two weeks' period), γ-irradiation (isotope $^{60}Co$ Artim Prague) at doses 0 Gy (control), 50 Gy and 100 Gy and microwave irradiation (microwave oven, P = 90 W) at doses 0 (control), 200 and 400 s (in two weeks' period). Potato tubers were irradiated 3 months after harvest. Buckwheat seedlings and plants were subjected to UV-A irradiation (high-pressure mercury vapour lamp, $\lambda = 365.5$ nm, P = 125 W) for 0 (control), 42 and 84 hours (in two weeks' period) and to γ-irradiation and microwave irradiation under the conditions as described above. After irradiation the plants were cultivated for one week and then dried in a drier at 50°C. Potato tubers were stored 2 months after irradiation at +5°C and then analysed.

**Preparation of buckwheat samples.** Seeds and dried plants were homogenised and extracted in Soxhlet apparatuses with 80% water ethanol solution for 20 hours. After adjusting of extract volume to 250 mL there were 5 mL aliquots pipetted into 50 mL volumetric flasks.

**Preparation of potato samples.** Tubers were homogenised and in the shortest time 10 g weighed into 100 mL volumetric flask and fulfilled with ethanol-water (80:20, V/V) up to the label. For the determination 5 mL aliquots were pipetted into 50 mL volumetric flasks.

**Determination of total polyphenols.** After dilution with distilled water to cca 30 mL volume it was 2.5 mL Folin-Ciocalteau's reagent p.a. (Nycom, Prague, CR) added. After agitation and 3 min standing 7.5 mL 20% $Na_2CO_3$ p.a. solution was added and the volume was adjusted to mark with distilled water. After thorough agitation and two hours standing absorbancy of blue solution in cuvettes 0.5 mL thickness at $\lambda = 765$ nm on Spekol 11 (Zeiss Jena, Germany) spectrophotometer was measured against blank. Polyphenol compounds were expressed as gallic acid content on dry matter (d.m.) basis. Two parallel determinations of each sample were performed.

**Dry matter determination.** The procedure was performed after Davídek et al. (1977). 10 g of fine-grained samples were dried to constant mass at 105°C.

**Determination of polyphenols and rutin by HPLC.** For more detail identification of polyphenolic compounds and rutin from ethanol-water (80:20, V/V) extracts was used HPLC with gradient elution (for phenolcarboxylic acids) and isocratic elution (for rutin) on Waters ™chromatograph (pump Waters ™600S, autosampler Waters ™717 plus, detector Waters ™PDA 996 – UV-VIS). For determination of phenolic acids in potato tubers was used column WATREX 250 × 4 mm Sepharon SGX C18 7 μm, mobile phase A – acetic acid-water (2:98, V/V), B – acetonitrile-acetic acid-water (30:2:68, V/V/V); gradient from 10 to 100% of phase B in phase A; elution time 25 min, flow rate 1.2 mL min$^{-1}$; injection 50 μL; detection at $\lambda = 280$ nm (Figure 1). As authentic samples caffeic acid p.a. (Fluka AG, Switzerland), ferulic acid p.a. (Fluka AG, Switzerland), chlorogenic acid p.a. (Merck, Darmstadt, FRG) and L-tyrosine p.a. (Company for chemical and metallurgical production, Ústí nad Labem, CR) were used. For analytical determination of rutin in potato tubers column WATREX 250 × 4 mm Sepharon SGX C18 7 μm, mobile phase A – acetic acid-water (5:495, V/V), B – methanol in ratio A:B = 55:45; elution time 22 min; flow rate 0.5 mL.min$^{-1}$, detection at $\lambda =$ 254 nm (Figure 2) was used. As standard rutin trihydrate p.a. (Fluka AG, Switzerland) was used.

**Determination of ascorbic acid.** Potato tuber was weighed after cleaning, homogenised with weighed amount of oxalic acid solution [28 g $(COOH)_2.2\,H_2O$ in 1 L]. The homogenate was filtered up and ascorbic acid was determined in the filtrate polarographically by the method of standard addition on the Eco-Tribo polarograph (Polaro-Sensors, Prague, CR) at these parameters: initial potential 250 mV, final potential 300 mV, rate 20 mV/s, bubble period 120 s, number of scans 1, static period 1 s, height of pulse 50 mV, width 80 mV. Two parallel determinations of each sample were performed.

## RESULTS AND DISSCUSSION

### Potato

In potato tubers (cv. Kordoba and cv. Rosella) the effect of different types of irradiation on ascorbic acid content (AA), the total polyphenol content (TP) and phenolic acids were investigated. Obtained results were statistically evaluated using *F*-test. From the variances obtained by *F*-test it was found that at the confidence level $p \leq 0.10$ the effect of UV-C irradiation on TP content in cv. Kordoba and cv. Rosella was not statistically different and the microwave irradiation on chlorogenic acid content and caffeic acid content, too. Similar effect had the γ-irradiation on caffeic acid content. The same trend was found for UV-C irradiation effect on ascorbic acid content and L-tyrosine.

Ascorbic acid content (AA) was determined as high as 300–680 mg.kg$^{-1}$ d.m. (in cv. Kordoba in av. 416 mg.kg$^{-1}$ d.m., in cv. Rosella in av. 526 mg.kg$^{-1}$ d.m.). These values (Table 1, Figure 3) correspond to the values found by many other authors (Duke 1992, Hamouz et al. 1999, Lachman et al. 2000a). UV-C and γ-irradiation caused the increase of AA (UV-C in av. by 31–5.5%, γ-irradiation in av. by 1–6%). AA as an antioxidant is involved in an antioxidant network and together with polyphenols scavenges free radicals that are originating by the effect of ionization by the action effect of irradiation. In the red variety Rosella a decrease of AA was not determined, because it contains anthocyanins belonging to the group of polyphenolic antioxidants, whereas the yellow coloured cv. Kordoba is much more susceptible to the intensive damage of tubers. In microwave irradiation an increase of AA content was determined. Microwave irradiation is not ionizing irradiation and can release AA from ascorbigen (Fragner et al. 1961, Šantavý et al. 1975).

TP content expressed as gallic acid in dry matter ranged in interval 3300–6200 mg.kg$^{-1}$ dry matter (Kordo-

Table 1. Content of ascorbic acid in potato tubers after irradiation (mg.kg$^{-1}$ dry matter)

| Variety | γ-irradiation | | | | | | Microwave irradiation | | | | | | UV-C irradiation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-γ | | 50 Gy | | 100 Gy | | C-micr. | | 200 s | | 400 s | | C-UV | | 42 h | | 84 h | |
| | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* |
| Kordoba | 509 | 9 | 534 | 6 | 294 | 7 | 295 | 3 | 521 | 9 | 304 | 4 | 682 | 6 | 307 | 7 | 295 | 5 |
| Rosella | 472 | 4 | 448 | 2 | 631 | 11 | 549 | 12 | 497 | 13 | 743 | 8 | 465 | 7 | 501 | 5 | 430 | 11 |

C-γ = control for γ-irradiation, C-micr. = control for microwave irradiation, C-UV = control for UV-C irradiation, avr = average, *s* = standard deviation; these controls were prepared and analysed at storage and time periods; the given doses of irradiation represent total summary doses applied in 14 days period

Table 2. Content of total polyphenols in potato tubers after irradiation expressed as gallic acid (mg.kg$^{-1}$ dry matter)

| Variety | γ-irradiation | | | | | | Microwave irradiation | | | | | | UV-C irradiation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-γ | | 50 Gy | | 100 Gy | | C-micr. | | 200 s | | 400 s | | C-UV | | 42 h | | 84 h | |
| | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* | avr | *s* |
| Kordoba | 3 700 | 25 | 4 350 | 28 | 5 300 | 57 | 4 850 | 30 | 3 600 | 31 | 4 550 | 42 | 3 000 | 32 | 3 950 | 28 | 6 200 | 54 |
| Rosella | 3 000 | 32 | 4 200 | 29 | 3 750 | 29 | 5 400 | 81 | 5 050 | 35 | 5 150 | 27 | 3 850 | 26 | 3 550 | 49 | 3 600 | 39 |

ba 4390 mg.kg$^{-1}$ d.m., Rosella 4150 mg.kg$^{-1}$ d.m.). UV-C and γ-irradiation caused the increase of phenolic compounds (Table 2, Figure 4). UV-C irradiation increased TP content at greater extent (by +10–44%) in comparison with γ-irradiation (by +25–33%). Microwave irradiation caused a TP decrease (in av. by 4–15%). The increase of TP content is induced as the reaction against UV-damage, the decrease of the activity of polyphenol oxidases and due to the change of the activity of phenylalanin ammonium-lyase (Ramamurthy et al. 1992). Among phenolic acids the most contained phenolic acids (Table 3, Figure 1) were amino acid L-tyrosine (in av. 2.25 mg.kg$^{-1}$ d.m.), chlorogenic acid (0.05 mg.kg$^{-1}$ d.m.), caffeic acid (0.007 mg.kg$^{-1}$ d.m.) and ferulic acid (0.004 mg.kg$^{-1}$ d.m.). Chen and Ho (1997) and Dao and Friedman (1992) found similar phenolcarboxylic acid pattern. UV-C irradiation caused the increase of L-tyrosine in cv. Kordoba (in av. by 150% at dose 42 h and by 2415% at dose 84 h), and in both varieties the increase of chlorogenic acid (+990%), caffeic acid (+250%) and ferulic acid (+97%) was found. Higher content of phenolcarboxylic acids was found in cv. Rosella (Table 2). γ-Irradiation increased L-tyrosine content (by 35–70%) and ferulic acid at 50 Gy dose, but at higher 100 Gy dose a decrease was found (–27%). Higher doses of γ-irradiation damaged the enzymic pathway of phenolcarboxylic biosynthesis (Ramamurthy et al. 1992). The same damage could be observed in the effect of microwave irradiation that destroys plant tissues by low heating-up associated with the damage of semi-permeability of cytoplasmatic membranes.

The richest source of polyphenolic compounds in seeds and green plants is *F. tataricum* Gärtner (11 400 mg.kg$^{-1}$ d.m. in seeds, 34 900 mg.kg$^{-1}$ d.m. in seedlings, 36 200 mg.kg$^{-1}$ d.m. in plants). Cv. Pyra contained in av. 4300 mg.kg$^{-1}$ d.m. in seeds, 14 400 mg.kg$^{-1}$ d.m. in seedlings, 24 100 mg.kg$^{-1}$ d.m. in plants, cv. Emka 4600 mg.kg$^{-1}$ d.m. in seeds, 15 700 mg.kg$^{-1}$ d.m. in seedlings and 12 400 mg.kg$^{-1}$ d.m. in plants (Table 4, Figure 5).

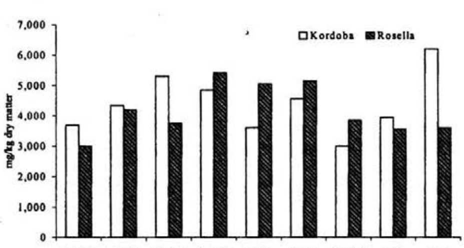


Figure 3. Changes of total polyphenol content in potatoes caused by different types of irradiation and their doses

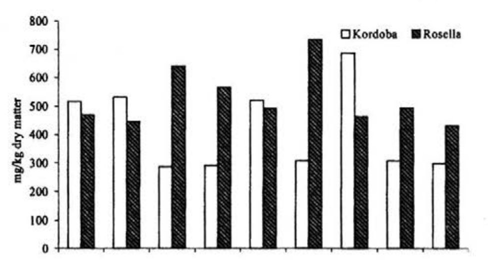


Figure 4. Changes of ascorbic acid content in potatoes caused by different types of irradiation and their doses

Table 3. Content of phenolic acids in potato tubers after irradiation (mg.kg$^{-1}$ dry matter)

| Acid | | Chlorogenic acid | | Ferulic acid | | L-Tyrosine | | Caffeic acid | |
|---|---|---|---|---|---|---|---|---|---|
| Variety | | Kordoba | Rosella | Kordoba | Rosella | Kordoba | Rosella | Kordoba | Rosella |
| C-γ | 0 Gy | 0.140 | 0.065 | 0.004 | 0.001 | 3.537 | 1.969 | 0.026 | 0.005 |
| γ-irrad. | 50 Gy | 0.021 | 0.010 | 0.008 | 0.002 | 3.725 | 3.715 | 0.004 | 0.004 |
| γ-irrad. | 100 Gy | 0.011 | 0.034 | 0.001 | 0.003 | 6.650 | 2.687 | 0.005 | 0.005 |
| C-micr. | 0 s | 0.266 | – | 0.002 | 0.001 | 4.205 | 1.459 | 0.035 | 0.002 |
| MW irrad. | 200 s | 0.015 | 0.018 | 0.001 | 0.001 | 3.215 | 1.434 | 0.003 | 0.001 |
| MW irrad. | 400 s | 0.033 | – | 0.002 | 0.001 | 2.634 | 0.774 | 0.006 | 0.001 |
| C-UV | 0 h | 0.008 | 0.007 | 0.007 | 0.003 | 0.090 | 0.917 | 0.002 | 0.004 |
| UV-C irrad. | 42 h | 0.036 | 0.003 | 0.001 | 0.019 | 0.229 | 0.681 | 0.007 | 0.000 |
| UV-C irrad. | 84 h | 0.103 | 0.059 | 0.007 | 0.009 | 2.254 | 0.473 | 0.013 | 0.009 |

UV-C irradiation increased TP content in av. by 13% (42 h) and 8% (84 h) in the seeds. The highest response was found in the tartarian buckwheat (the increase by 16–18%) and cv. Emka (by 19%), whereas in cv. Pyra significant differences were not found (Table 5). In the buckwheat seedlings and plants it was not used UV-C irradiation because of massive damage and destruction of plants. Instead UV-A irradiation was used that increased TP content in full-grown plants in av. by 20% in cv. Emka, by 44–48% in *F. tataricum* Gärtner and by 39–52% in cv. Pyra. γ-Irradiation at lower doses increased moderately TP content, but high dose had already diminishing effect (in cv. Pyra by 4–19%, cv. Emka by 19–40%). The TP decrease by 20% was estimated in seedlings (the highest in cv. Pyra by 28%, in cv. Emka by 8–14%). In full-grown plants the significant TP decrease was recorded in cv. Pyra (by 84–92%) and tartarian buckwheat (by 5.5–32%). γ-Irradiation caused retarding of the growth of seedlings and plants. Microwave irradiation had in consequence significant changes in seeds, but in plants the doses had to be diminished from 200 s and 400 s to 20 s and 40 s regarding heavy damage to plant tissues at higher doses. In all investigated plants the significant TP decrease was observed (in *F. tataricum* Gärtner by 77%, in cv. Pyra by 69% and cv. Emka by 57%).

Rutin is a major flavonoid compound contained in buckwheat (Figure 2, Lachman et al. 1998b). The highest source of rutin is tartarian buckwheat (10 700 mg.kg$^{-1}$ d.m. in seeds, 39 150 mg.kg$^{-1}$ d.m. in seedlings and 25 400 mg.kg$^{-1}$ d.m. in plants) in comparison with cv. Pyra (300 mg.kg$^{-1}$ d.m. in seeds, 2600 mg.kg$^{-1}$ d.m. in seedlings and 12 600 mg.kg$^{-1}$ d.m. in plants) and cv. Emka (250 mg.kg$^{-1}$ d.m. in seeds, 5100 mg.kg$^{-1}$ d.m. in seedlings and 4200 mg.kg$^{-1}$ d.m. in plants). The effects of irradiations are summarized in Table 5. UV-C irradiation enhanced the content of rutin in av. by 3% (in cv. Emka by 37%, cv. Pyra by 5%). UV-A irradiation enhanced the content of rutin in full-grown plants in av. by 48% (in cv. Pyra by 224%, in cv. Emka by 72%, in *F. tataricum* Gärtner by 10%). These results confirm the hypothesis that UV irradiation has a stimulating effect on the accumulation of rutin in green overground buckwheat biomass (Scherf and Zenk 1967). γ-Irradiation at lower doses enhanced the content of rutin in seedlings (in cv. Pyra by 320% at 50 Gy and by 106% at 100 Gy, in tartarian buckwheat by 25%, in cv. Emka by 32% at 50 Gy). The higher doses had already a depressive effect on the content of rutin because its level decreased in av. by 59% in comparison with the control. The decline effect was also observed in seeds (in cv. Pyra by 16%, in *F. tataricum* Gärtner by 5%) and the significant decrease was found in plants (in cv. Emka by 30%, in cv. Pyra by 97% or 85%) with the exception of tartarian buckwheat, where the increase was estimated (by 32% at 50 Gy and 19% at 100 Gy). Microwave irradiation induced a decrease of rutin in plants (in cv. Pyra, *F. tataricum* Gärtner and cv. Emka by 43%, 35% and 26%, resp.). The changes of the content of rutin in the seeds of the investigated cultivars were different (in cv. Pyra an increase by 38% was determined, in tartarian buckwheat by 4%, in cv. Emka in contrary a decline by 65% was found).

Table 4. Content of total polyphenols and rutin buckwheat (mg.kg$^{-1}$ dry matter)

| Variety | Seeds | | Seedlings | | Plants | |
|---|---|---|---|---|---|---|
| | TP | rutin | TP | rutin | TP | rutin |
| Tartarian buckwheat | 11 400 | 10 700 | 34 900 | 39 200 | 36 200 | 25 450 |
| Pyra | 4 350 | 300 | 14 400 | 2 600 | 24 150 | 12 600 |
| Emka | 4 600 | 250 | 15 700 | 5 150 | 12 400 | 4 200 |

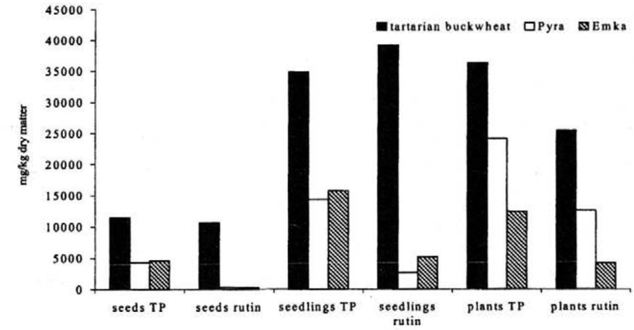


Figure 5. Content of rutin and total polyphenols in buckwheat and tartarian buckwheat

## CONCLUSIONS

UV-C, γ- and microwave irradiation affected the content of secondary metabolites, such as polyphenols or ascorbic acid in plants. UV-C and γ-irradiation caused the increase of polyphenolic compounds in potato tubers (cv. Kordoba and cv. Rosella). UV-C irradiation increased TP content at greater extent in comparison with γ-irradiation. In contrary, microwave irradiation caused the TP decrease. The increase of TP content is a response to UV-C and γ-irradiation damage and wounding regarding the activation of plant defence system. Similar pattern was found for phenolcarboxylic acids (L-tyrosine, chlorogenic acid, caffeic acid and ferulic acid). There are differences in cultivars. γ-Irradiation activates the defence mechanism at lower doses, but at higher doses is the enzymic defence mechanism producing polyphenols already damaged. The same damage effect was observed in the effect of microwave irradiation. UV-C and γ-irradiation caused the decrease of ascorbic acid content, because ascorbic acid is an antioxidant involved in the antioxidant network of plants. The red variety Rosella containing anthocyanins (efficient antioxidants) had the content of ascorbic acid saved. Microwave irradiation as not ionizing irradiation can liberate ascorbic acid from ascorbigen and it increased its content. UV-C and γ-irradiation (at lower doses) enhanced TP content in buckwheat (in buckwheat plants UV-A irradiation was used). High γ-irradiation doses diminished total polyphenol content due to the destructive effect. Due to the smashing effect of microwave irradiation – the TP decrease was found. Also UV-C irradiation had a stimulating effect on the accumulation of rutin in full-grown plants. The same effect was observed in γ-irradiation at lower doses, but at higher doses in contrary TP content decreased. There were found cultivar differences – tartarian buckwheat was much more resistant to UV-C and γ-irradiation enhancing rutin content in comparison with buckwheat cv. Pyra and cv. Emka. Microwave irradiation induced in plants a decrease of rutin, whereas in plants the responses were different.

In general, UV-C and UV-A irradiation increased TP, phenolcarboxylic acid and rutin content and decreased AA content. γ-Irradiation at lower doses had the same effect, but at higher doses the content of phenols, flavonoids and AA decreased. Microwave irradiation due to the thermical damage of biological enzymic systems decreased TP, phenolcarboxylic acid and rutin content. AA content increased because of its liberating from ascorbinogen.

Table 5. Content of total polyphenols and rutin in buckwheat influenced by irradiation (mg.kg$^{-1}$)

| Organ | Dose of irradiation | Tartarian buckwheat | | Pyra | | Emka | |
|---|---|---|---|---|---|---|---|
| | | TP | rutin | TP | rutin | TP | rutin |
| Seedlings | γ 0 Gy | 32 550 | 38 800 | 17 550 | 1 100 | 17 000 | 5 650 |
| | γ 50 Gy | 40 950 | 29 800 | 12 500 | 4 600 | 14 600 | 7 500 |
| | γ 100 Gy | 31 150 | 48 900 | 13 100 | 2 250 | 15 550 | 2 300 |
| Plants | γ 0 Gy | 47 250 | 19 500 | 73 450 | 28 500 | 10 000 | 4 700 |
| | γ 50 Gy | 31 700 | 25 900 | 11 350 | 750 | 12 450 | 4 850 |
| | γ 100 Gy | 44 600 | 23 400 | 2 000 | 4 050 | 13 400 | 3 300 |
| Plants | UV-A 0 h | 36 900 | 32 700 | 9 200 | 1 650 | 18 100 | 3 300 |
| | UV-A 42 h | 54 700 | 35 950 | 13 950 | 2 800 | – | – |
| | UV-A 84 h | 52 200 | 35 150 | 12 800 | 5 700 | 15 550 | 5 650 |
| Plants | MW 0 s | 18 200 | 18 850 | 14 600 | 6 150 | 13 400 | 3 900 |
| | MW 20 s | – | – | 15 400 | 5 800 | 10 650 | 5 050 |
| | MW 40 s | 4 100 | 12 100 | 4 500 | 3 450 | 5 700 | 2 850 |

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**ABSTRAKT**

**Změny vybraných sekundárních metabolitů v bramborách a pohance způsobené UV, γ- a mikrovlnným zářením**

Byly sledovány změny v obsahu celkových polyfenolů, fenolkarboxylových kyselin a askorbové kyseliny v bramborových hlízách odrůd Kordoba a Rosella a u tří odrůd pohanky (semena, naklíčená semena a rostliny, *F. esculentum* Moench odrůdy Pyra a Emka a *F. tataricum* Gärtner) vyvolané UV-C zářením ($\lambda$ = 253,7 nm, P = 75 W, 0 – kontrola, 42 a 84 h), γ-zářením ($^{60}Co$ Artim Praha v dávkách 0 – kontrola, 50 Gy a 100 Gy) a mikrovlnným zářením (P = 90 W, 0 – kontrola, 200 s a 400 s). Brambory obsahovaly průměrně 300–680 $mg.kg^{-1}$ askorbové kyseliny v sušině. Vlivem UV-C a γ-záření docházelo k poklesu obsahu askorbové kyseliny v sušině hlíz, vlivem mikrovlnného záření k nárůstu až o 78 %. Obsah celkových fenolických látek v sušině hlíz brambor činil 3300–6200 $mg.kg^{-1}$. Fenolické látky brambor byly identifikovány metodou HPLC s UV detekcí. Jako nejvíce zastoupená látka se ukázala fenolická aminokyselina L-tyrozin (2,25 $mg.kg^{-1}$ sušiny), dále chlorogenová kyselina (0,05 $mg.kg^{-1}$ sušiny), kávová kyselina (0,007 $mg.kg^{-1}$ sušiny) a ferulová kyselina (0,004 $mg.kg^{-1}$ sušiny). Účinkem mikrovlnného záření došlo k poklesu všech kyselin; γ-záření mělo za následek pokles všech sledovaných kyselin (o 68–85 %) mimo L-tyrozin, jehož obsah se zvýšil (o 35–70%); UV-C záření způsobilo pokles L-tyrozinu u odrůdy Rosella, ale nárůst u odrůdy Kordoba. U ostatních kyselin se obsah zvyšoval. Pohanka tatarská (*F. tataricum* Gärtner) obsahovala mnohem vyšší hladinu celkových polyfenolů a rutinu oproti pohance seté (*F. esculentum* Moench). S růstem a vývojem rostliny se zvyšuje obsah celkových polyfenolů a rutinu. Ozářením semen UV-C zářením docházelo k nárůstu celkových polyfenolů a rutinu, γ-zářením docházelo při nižších dávkách k nárůstu celkových polyfenolů, ale vyšší dávky měly opačný účinek. Podobně se chovala i hladina rutinu, která se γ-zářením snižovala. Mikrovlnné záření neovlivňovalo množství celkových polyfenolů, ale působilo zvýšení rutinu. Pouze u odrůdy Emka došlo k poklesu. Naklíčená semena a rostliny vlivem γ-záření snižovaly obsah celkových polyfenolů i rutinu. Naklíčená semena reagovala na γ-záření přechodným zvýšením rutinu, ale při vyšších dávkách jeho obsah klesal. UV-A záření u vzrostlých rostlin zvyšovalo hodnotu celkových polyfenolů i rutinu. Mikrovlnné záření mělo za následek pokles celkových polyfenolů i rutinu.

**Klíčová slova:** brambory; pohanka; polyfenoly; askorbová kyselina; fenolkarboxylové kyseliny; rutin; UV-C a UV-A záření; γ-záření; mikrovlnné záření

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# Baking quality in the protein lines of wheat land races and obsolete varieties

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**ABSTRACT**

By means of gluten quality ($T_0$, $Q_0$) test, SDS sedimentation test and mixograph, the baking quality in 37 protein lines isolated from 24 land races and obsolete winter wheat varieties were evaluated by protein genetic markers, i.e. gliadins (SGE) and HMW glutenin subunits (SDS PAGE). A selection of genotypes, lines with higher parameters of indirect determination of baking quality according to gluten quality and sedimetation value was accomplished. At the same time, significant to highly significant differences among sister protein lines in varieties heterogenous in the composition of gliadins and HMW glutenin subunits in gluten quality and sedimentation value were proved. Rheological characters of the evaluated collection of varieties, protein lines, were determined by the mixograph. There were selected genotypes, lines with optimum mixograph parameters. Simultaneously, there were confirmed, by means of *t*-test, significantly to highly significantly improved mixograph properties in some protein lines as compared with the Samanta control variety. A significant positive correlation was found between sedimentation value as the most reliable index of indirect determination of baking quality (Lelley 1976) and mixograph dough stability. A highly significant correlation dependence was proved between sedimentation value and mixograph dough elasticity. Vertical electrophoretic analysis of gliadin genetic markers in starch gel (SGE) and electrophoresis of HMW glutenin subunits (SDS PAGE) enable to isolate protein lines significantly or highly significantly differing in gluten quality test ($T_0$, $Q_0$) or SDS sedimentation test values and in some rheological properties determined by mixograph from genetically heterogenous population varieties. Isolated protein lines present more suitable parent forms in hybridization breeding programmes than the heterogenous initial ones.

**Keywords:** wheat; land races; obsolete varieties; electrophoresis; protein line; baking quality

Signal genes determining gliadin and glutenin high-molecular proteins of wheat caryopses enable markering the baking quality, rust and cold resistance (Sozinov 1985). Individual sister protein lines selected from populations of land races and obsolete varieties can differ in markered properties (Šašek et al. 1985). The objective of the paper is to assess the baking quality in wheat land races and obsolete wheat varieties, or, as the case may be, of their protein lines separated by means of electrophoretic analysis of gliadins and HMW glutenin subunits.

## MATERIAL AND METHODS

A collection of 100 land varieties and old wheat cultivars from the assortment of the Gene Bank at the Research Institute of Crop Production Prague-Ruzyně was evaluated by means of protein genetic markers (Černý and Šašek 1996). Out of this collection, 37 protein lines isolated from 24 land varieties and old wheat cultivars were tested for the determination of baking quality.

The field experiment was established in 1995 by sowing 37 protein lines of evaluated land races and obsolete wheat cultivars in four repetitions in one-row plots 2 m long with 25 cm distance at the Demonstration and Experimental Field of the Czech University of Agriculture in Prague. With regard to the application of growth retardants, the control variant, i.e. the Samanta cultivar, was sown separately outside the experiment.

The applied procedures of gliadin and HMW glutenin electrophoreses and methods of separation of allelic blocks from electrophoretic spectra were published (Černý and Šašek 1996).

The baking quality was assessed in four replicates by determining the sedimentation (according to the standard PN 232/93, SDS sedimentation test, ZNZ Praha) and by gluten quality test, determining the swelling ($Q_0$) and stretchiness ($T_0$) properties (according to H. Perten in wheat meal and wheat flour, ISS Standard 155, AAC standard 3812).

Using the Swanson and Working Mixograph (Nat. Hfg. Co. Lincol) the following values were determined: dough development time (DDT), dough stability, i.e. the time and dough consistency higher than 500 mixograph units (MU) (stability = STAB), maximum curve peak (P), tolerance index, i.e. difference between the upper part of the curve at its maximum and the upper part of the curve 5 min after reaching the peak (mixing tolerance index = MTI) and dough elasticity expressed by the degree of decrease of dough consistency below the level of 500 MU 12 min after reaching the curve peak (elasticity = E).

Standard Mixograph test 10 g samples according to Hanišová et al. (2000) was used for mixograph resistance.

The obtained data were assessed by $t$-test of different means or by selection above or below the limit of the evaluated collection of lines $\bar{x}$ +/– 1$s$ and $\bar{x}$ +/– 2$s$, respectively.

## RESULTS AND DISCUSSION

Most of the European wheat breeders are routinely using HMW glutenin subunits as markers of the baking quality. At the present, the research of LMW glutenin subunits as alternative markers of baking quality is continuing. There has been renewed also the interest in the application of gliadin markers of baking quality (Johansson 1996).

The level of heterogeneity in wheat land races was studied by Schmid et al. (1988) by means of electrophoretic gliadin analysis on polyacrylamide gel (PAGE). In some cases they proved the heterogeneity in gliadin composition in evaluated Swiss land spring wheat varieties, markering the overall genetic heterogeneity in these varieties – populations.

A model of electrophoretic gliadin analysis as markers of genetic structure of varieties was implemented by Manev and Stehno (1997) by A-PAGE ISTA in two Czech wheat land races i.e. Mandelíková ratbořská Mara and Červená perla. The obtained results correspond with our findings (Černý and Šašek 1996) with the Mandelíkova ratbořská Mara variety as a two-line variety and Červená perla as a single-line one.

Gliadin electrophoresis of 9 winter land races performed by SGE (Černý and Šašek 1996) and A-PAGE ISTA (Manev 1999) produced different results in the varieties Radošínská Dorada and Hanácká osinatá. It was found by SGE that the both mentioned land races present populations composed of two main gliadin lines. It was not confirmed by A-PAGE ISTA.

So far with reference method A-PAGE ISTA, there were not separated and determined basic allelic gliadin blocks enabling genetic interpretation of gliadin electrophoretic spectra. Possibilities of genetic interpretation of gliadin electrophoretic spectra obtained by A-PAGE ISTA were outlined by Šašek and Černý (1995).

Homogeneity and homozygosity in evaluated protein lines in the collection of 24 varieties were assessed by electrophoretic gliadin and HMW glutenins analyses in 1994 and 1995.

The obtained results agree with the great protein polymorphism of the evaluated collection of 24 land races and obsolete wheat cultivars reaching 30 per cent. The highest level of protein polymorphism was found in the varieties Valtická osinatá (4 lines), Hodonínská univerzální and Hořická (3 lines).

Polymorphism in the electrophoretic composition of HMW glutenins is lower than in gliadins (Černý and

Table 1. Selected varieties, lines with gluten quality and sedimentation values higher than $\bar{x}$ + 1$s$ or $\bar{x}$ + 2$s$ in the evaluated collection of 37 protein lines

| Criterion of the baking quality | Values of the set of 37 lines (S) and control variety (C) | | | Selected lines | Origin variety |
|---|---|---|---|---|---|
| | $\bar{x}$ | $s$ | $s_{\bar{x}}$ | | |
| $Q_o$ (S) | 4.49 | 4.47 | 0.78 | $\bar{x}$ + 1$s$ = 9.26 | |
| | | | | č. 33 | Valtická osinatá |
| | | | | č. 16 | Kelčanská vouska |
| | | | | č. 34 | Valtická osinatá |
| | | | | č. 35 | Valtická osinatá |
| | | | | č. 32 | Valtická osinatá |
| | | | | č. 13 | Hořická |
| (C) | 7.5 | 2.12 | 1.50 | | |
| $T_o$ (S) | 15.52 | 3.40 | 0.56 | $\bar{x}$ + 1$s$ = 18.92 | |
| | | | | č.10 | Hodonínská univerzální |
| | | | | č. 21 | Postoloprtská přesívka |
| | | | | č. 15 | Kaštická přesívka |
| | | | | č. 1 | Bučanská červenoklasá |
| (C) | 14.29 | 2.12 | 1.50 | | |
| SDS ST (S) | 42.29 | 12.16 | 2.00 | $\bar{x}$ + 2$s$ = 66.61 | |
| | | | | č. 14 | Hospodářská bezosinná |
| | | | | č. 17 | Kelčanská vouska |
| | | | | č. 29 | Slovenská 777 |
| (C) | 76.60 | 1.94 | 0.86 | | |

S = set of selected lines, C = control variety Samanta, $Q_o$ = swelling capacity of gluten, $T_o$ = stretchiness of gluten, SDS ST = sedimentation

Table 2. Significantly different means of criteria of baking quality in sister protein lines of polymorphous varieties

| Variety | Criterion | Entry N | Line | $\bar{x}$ | $s$ | $s_{\bar{x}}$ | Significant difference between lines |
|---|---|---|---|---|---|---|---|
| Dregerova | $Q_0$ | 3 | B | 1.5 | 1.0 | 0.5 | |
| | | 4 | A | 3.5 | 1.0 | 0.5 | 3–4* |
| Hodonínská | SDS ST | 8 | Ba | 53.5 | 1.0 | 0.5 | |
| | | 9 | Ab | 25.75 | 2.06 | 1.03 | 8–10** |
| | | 10 | A | 26.5 | 1.29 | 0.64 | 8–9** |
| Hořická | SDS ST | 11 | A | 34.5 | 1.29 | 0.14 | 11–13** |
| | | 12 | B | 39.0 | 3.56 | 1.78 | 12–13** |
| | | 13 | D | 47.25 | 0.96 | 0.48 | |
| Kelčanská | SDS ST | 16 | A | 52.0 | 3.37 | 1.68 | 16–17** |
| | | 17 | B | 64.8 | 3.90 | 1.05 | |
| Pavlovická | $Q_0$ | 19 | B | 4.50 | 0.58 | 0.29 | |
| | | 20 | A | 2.25 | 0.15 | 0.48 | 19–20** |
| Valtická osinatá | SDS ST | 32 | Ba | 47.75 | 2.22 | 1.11 | 34–33** |
| | | 33 | Ca | 54.75 | 1.26 | 0.63 | 34–35** |
| | | 34 | Db | 59.25 | 0.48 | 0.48 | 34–36** |
| | | 35 | Aa | 44.75 | 1.71 | 0.85 | 34–32** |
| | | 36 | A | 49.0 | 1.4 | 0.7 | 33–35** |
| | | | | | | | 33–36** |
| | | | | | | | 33–32** |
| | | | | | | | 35–36** |
| Valtická osinatá | $Q_0$ | 32 | Ba | 12.0 | 1.4 | 0.99 | 32–33** |
| | | 33 | Ca | 16.75 | 0.86 | 0.61 | 32–34* |
| | | 34 | Db | 15.75 | 1.71 | 1.21 | 32–35* |
| | | 35 | Aa | 15.25 | 1.5 | 1.07 | 32–36* |

SDS ST = sedimentation (ml), $Q_0$ = swelling capacity, N = number of the line
* significant at $P_{0.05}$, ** significant at $P_{0.01}$

Šašek 1996). In the original collection of 100 land races and obsolete wheat varieties it reached only 4 per cent. In the evaluated collection of 24 varieties polymorphism of HMW glutenins manifested itself in the Hodonínská univerzální and Valtická osinatá varieties wich makes 8 per cent.

According to individual criteria of indirect determination of baking quality (gluten quality [$T_0$, $Q_0$] test, SDS ST) the potential gene resources or protein lines were selected exceeding the values $\bar{x}$ +/– 2*s* or $\bar{x}$ +/– 1*s* of the evaluated collection.

Selected varieties, lines are characterized in Table 1. Selected varieties, lines exceeding the values in the used criteria $\bar{x}$ +/– 1*s* or $\bar{x}$ +/– 2*s* of the evaluated collection were compared with the Samanta control variety by means of *t*-test. In none of the used criteria the selected varieties reached significantly higher values than the control.

In polymorphic population varieties the protein polymorphism can marker polymorphism of economic traits, including baking quality. For the assessment of differences in used criteria of indirect evaluation of baking quality by gluten quality ($T_0$, $Q_0$) and SDS sedimentation tests the sister lines of protein polymorphic varieties were tested by *t*-test of different means (Table 2).

The determined statistically significant differences in sedimentation value and swelling capacity in sister protein lines of selected protein polymorphic varieties (Dregerova, Hodonínská univerzální, Hořická, Kelčanská, Pavlovická, Valtická osinatá) are in favour of the benefit of electrophoresis of gliadins and HMW glutenins as genetic markers characterizing not only the polymorphism of reserve proteins but also the bound polymorphism in many economically important traits.

For hybridization programmes it is more advantageous not to use the polymorphic variety – population as a parent component but to apply its individual protein lines with defined markered characters.

The rheological properties were assessed in varieties, their protein lines were evaluated by selected mixograph criteria (Pomeranz 1988).

Mixograph measures and records dough resistance to mixing. The resulting curve characterizes the optimum time of dough development, stability and futher characters.

Analogically as with the evaluation of glutens and sedimentation, there were isolated varieties, lines with mixograph values higher than $\bar{x}$ +/– 1*s* or $\bar{x}$ +/– 2*s* (Table 3) of the assessed collection of genotypes of the whole collection. The selected genotypes were further compared by *t*-test with the Samanta control variety (Table 4).

Some mixograph criteria included ability of protein lines of wheat land races to marker the statistically significant-

Table 3. Selected varieties, lines with mixograph values higher than $\bar{x}$ + 1$s$ or $\bar{x}$ + 2$s$ in the evaluated collection of 33 protein lines or with values lower than $\bar{x}$ – 1$s$ or $\bar{x}$ – 2$s$

| Mixograph criterion | Values of the set of 33 lines, varieties | Selected lines | Origin, variety |
|---|---|---|---|
| DDT (sec) | $\bar{x} - 2s = 62.02$ | č. 27 | Šumavská |
| | $\bar{x} - 1s = 79.76$ | č. 9 | Hodonínská osinatá |
| | | č. 30 | Valtická osinatá |
| | | č. 32 | Valtická osinatá |
| STAB (sec) | $\bar{x} + 1s = 486$ | č. 12 | Hořická |
| | | č. 13 | Hořická |
| | | č. 7 | Hodoninská osinatka |
| | | č. 8 | Hodonínská univerzální |
| | | č. 30 | Valtická osinatá |
| | | č. 31 | Valtická osinatá |
| P (MU) | $\bar{x} + 2s = 6.48$ | č. 7 | Hodoninská osinatka |
| | | č. 8 | Hodonínská univerzální |
| | | č. 14 | Hospodářská bezosinná |
| | | č. 25 | Slovenská 2000 |
| | | č. 33 | Židlochovická |
| MTI (MU) | $\bar{x} + 1s = 0.78$ | č. 29 | Valtická osinatá |
| | | č. 31 | Valtická osinatá |
| | | č. 32 | Valtická osinatá |
| E (MU) | $\bar{x} + 1s = 1.63$ | č. 3 | Dregerova |
| | | č. 8 | Hodonínská univerzální |
| | | č. 12 | Hořická |
| | | č. 14 | Hospodářská bezosinná |
| | | č. 31 | Valtická osinatá |

DDT = dough development time, STAB = dough stability, P = maximum curve peak, MTI = mixing tolerance index, E = elasticity, MU = mixograph unit, sec.= second

ly improved properties in comparison with the Samanta control variety that belongs to the class A (a quality bread wheat).

The dough development time became significantly shorter in the lines No 27 (originating from the Šumavská landrace) and No 30 (originating from the Valtická osinatá

Table 4. Mixograph criteria of selected genotypes (lines, varieties) and control Samanta variety ($t$-test)

| Criterion | Selected line, or control variety (C) | $\bar{x}$ | $s$ | $t$-test, significance |
|---|---|---|---|---|
| DDT (sec) | C | 180 | 14.14 | |
| | č. 27 (Šumavská) | 60 | 14.14 | 6* |
| | č. 30 (Valtická osinatá) | 70 | 14.14 | 5.5* |
| STAB (sec) | C | 320 | | |
| | č. 7 (Hodonínská osinatka) | 612.5 | 38.89 | 6* |
| | č. 8 (Hodonínská univerzální) | 555.0 | 7.07 | 9.14* |
| | č. 12 (Hořická) | 560.0 | 42.03 | 4.7* |
| | č. 13 (Hořická) | 617.5 | 60.1 | 4.48* |
| P (MU) | C | 5.8 | 0.28 | |
| | č. 7 (Hodonínská osinatka) | 7.6 | 0.28 | 4.5* |
| MTI (MU) | C | 0.68 | 0.05 | |
| | č. 31 (Valtická osinatá) | 0.15 | 0.07 | 5.89* |
| | č. 32 (Valtická osinatá) | 0.1 | 0.01 | 13.4** |
| E (MU) | C | 1.7 | 0.07 | |
| | no significantly differences between the control and evaluated lines | | | |

DDT = dough development time, STAB = dough stability, P = maximum curve peak, MTI = mixing tolerance index, E = elasticity, MU = mixograph unit, sec = second, C = control variety Samanta

* significant at $P_{0.05}$, ** significant at $P_{0.01}$

Table 5. Correlation among selected mixograph properties and sedimentation value of the SDS seditest

| | Value correlation coefficient $r$ | Significance |
|---|---|---|
| DDT | 0.24 | |
| STAB | 0.37 | * |
| P | 0.22 | |
| MTI | 0.30 | |
| E | 0.48 | ** |

DDT = dough development time, STAB = dough stability, P = maximum curve peak, MTI = mixing tolerance index, E = elasticity, SDS ST = SDS sedimentation test
* = significant at $P_{0.05}$, ** = significant at $P_{0.01}$

variety), but on the other hand, the dough stability increased in the lines No 7 and 8 (originating from the Hodonínská osinatá variety) and in the lines No 12 and 13 (originating from the Hořická variety). Significantly higher peak values of the mixograph curve as compared with the control variety reached the line No 7 originating from the Hodonínská osinatá variety. A significantly more suitable value of tolerance index (MTI) was found in the lines No 31 and 32 isolated from the Valtická osinatá variety.

For the assessment of the values of individual mixograph indices as compared with the SDS sedimentation test, the correlation analysis was used. Results of this analysis are presented in Table 5.

According to the results of the correlation analysis a highly significant correlation was found between the sedimentation value obtained by the SDS ST and dough elasticity, i.e. the level of decrease of dough strength below 500 MU 12 hours after reaching the peak of the mixograph curve.

A significant correlation was found between the sedimentation value and stability, i.e. period of dough above the level of 500 MU. A higher dough stability was proved in altogether 4 evaluated protein lines, as it is evident from the Table 4.

By means of electrophoresis of applied protein genetic markers it is also possible to determine significant differences in mixograph properties of various protein lines isolated from population varieties (Table 5).

Significant to highly significant differences amog sister lines were manifested dough stability and dough elasticity especially in mixograph. The determined heterogeneity in land races or obsolete wheat varieties in mixograph properties confirms the advantage of selecting protein lines from these population varieties by means of electrophoresis of protein genetic markers. Instead of populations of sister lines of heterogenous varieties the hybridization programs can apply homogenous and homozygous lines with defined genotypes determining the baking quality and some important rheological dough properties.

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## ABSTRAKT

**Pekařská jakost bílkovinných linií krajových a starých odrůd pšenice seté**

Stanovením tažnosti ($T_o$) a bobtnavosti ($Q_o$) lepku a pomocí sedimentačního testu a mixografu byla hodnocena pekařská jakost 37 bílkovinných linií, vyčleněných z 24 krajových a starých šlechtěných odrůd ozimé pšenice elektroforézou bílkovinných genetických markerů, tj. gliadinů (SGE) a podjednotek VMH gluteninů (SDS PAGE). U odrůd heterogenních ve skladbě sledovaných bílkovinných genetických markerů byly prokázány významné až vysoce významné rozdíly v jakosti lepku a v sedimentační hodnotě. Byly získány linie s optimálními mixografickými parametry. Pomocí $t$-testu byly vybrány bílkovinné linie významně až vysoce významně lepší než kontrolní odrůda Samanta. Byla zjištěna významná kladná korelace mezi sedimentační hodnotou a mixografickou stabilitou těsta a vysoce významná kladná korelace mezi sedimentační hodnotou a mixografickou pružností těsta. Vertikální elektroforéza gliadinů ve škrobovém gelu (SGE) a elektroforéza podjednotek VMH gluteninů (SDS PAGE) umožňují vyčlenit z odrůd – populací bílkovinné linie lišící se významně či vysoce významně v hodnotách jakosti lepku, v sedimentační hodnotě a v některých mixografických ukazatelích. Vyčleněné bílkovinné linie představují v hybridizačních programech vhodnější rodičovské formy než výchozí odrůdy – populace.

**Klíčová slova:** pšenice setá; krajové odrůdy; staré šlechtěné odrůdy; elektroforéza; bílkovinné linie; pekařská jakost

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# The need of supplemental irrigation in the Czech Republic

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**ABSTRACT**

A retrospective analysis was used to determine the compensation of moisture requirements of potatoes, winter wheat, sugar beet and hops by rainfall in two typical areas of CR where irrigation systems were built and privatized: Poohří (Žatec station of the Czech Hydrometeorological Institute - CHMI) and the lower Elbe (Doksany station of CHMI). Moisture requirements of the above crops in 1996–2000 were calculated by the method of biological curve. Total water deficits in 1997–2000 were found to amount nearly to the total moisture requirement of crops defined in the Czech standard ČSN 75 0434 *Water Requirements for Supplemental Irrigation* or they were even higher. They were highest at Žatec in 2000, when they exceeded the total moisture requirement of wheat by 32 mm, sugar beet by 61 mm, early potatoes by 90 mm and hops even by 113 mm.

**Keywords:** drought; retrospective water balance; supplemental irrigation

Yields of farm crops in the most productive regions of CR are demonstrably influenced by rainfall deficits, by spells of drought. In accordance with the description of climatic regions in Order No. 327/1998 of the Ministry of Agriculture laying down the characteristics of assessed soil ecological units and the procedure of their recording and updating, the privatized medium- and large-size irrigation systems are located in climatic regions 0 (very warm, dry) and 1 (warm, dry). The irrigable area in successfully privatized irrigation systems is about 135 000 ha.

There is a high probability of occurrence of crop moisture deficits, spells of drought, in the irrigation areas of CR. The rainfall amount with 50% frequency of occurrence does not compensate moisture requirements of major field crops, orchards and hops, or the rainfall distribution throughout the growing season is not suitable. It increases farming risk on lands with high production potential, high site class and high official land price derived from it.

Spell of drought is a contingent disastrous natural phenomenon afflicting extensive lands on which crop growth and ontogenesis, and biomass production and accumulation are restricted. Drought caused by a deficit of natural rainfall results in the passive hydrological balance in watersheds and in the soil.

Drought is a disaster, with stochastic occurrence and effects. The amount of natural rainfall, its distribution in area and time, concomitant high air temperatures, low relative atmospheric humidity, high evaporation rate are the basic mensurable parameters for drought indication and quantification in the given landscape and for evaluation of its effect on the agrosystem development.

If the values of water output, evapotranspiration, are substantially higher than water input, amounts of effective rainfall, the consequences of crop water deficits are displayed. The instantaneous reserve of soil water in the soil profile rhizosphere does not guarantee the fluent supply of water to plants corresponding to the values of potential evapotranspiration. Soil drought occurs, depending on the hydropedological properties of soil profile, on the effective reserve of soil water.

Soil drought causes restrictions of the water status of plants, physiological and biochemical processes, and finally, a reduction in biomass production, yield depression. The potential yield capacity of soils is not exploited, the cultures are depreciated. Supplemental irrigation is an efficient regulatory measure that can eliminate the negative impacts of drought.

Irrigation of supplemental type in CR based on the contingent occurrence of rainfall hard to forecast, variations in moisture requirements of crops during their ontogenesis, require an operative, regional-level evaluation of the compensation of crop moisture requirements in real time. The results of water balance are used to calculate the irrigation water requirement, to control the irrigation schedule for each crop, inserted hydropedological properties of soil and the system of irrigation water distribution to the cropping area (irrigation method).

The effect of irrigation water supplies on crop yield formation is a determinative qualitative criterion of irrigation exploitation in the farming system. Irrigation is a caused cultural practice that has to control the water regime of soils according to actual moisture requirements of crops.

The need of supplemental irrigation and its qualified exploitation, yield efficiency of irrigation water in the climatic conditions of CR are demonstrated by empirical experience and documented by numerous scientific and technical papers (Penka et al. 1973, Šimon 1995, Slavík 1996, 1997, 1998, 2000, Slavík and Zavadil 1999, Zavadil 2000 etc.).

## MATERIAL AND METHODS

A retrospective analysis was used to determine the compensation of moisture requirements of some crops – potatoes, winter wheat, sugar beet and hops by rainfall. Two typical areas with built and privatized irrigation systems were selected: Poohří (Žatec Station of the Czech Hydrometeorological Institute – CHMI) and the lower Elbe (Doksany Station of CHMI). Data over a five-year period of observation were processed (1996–2000).

Crop moisture requirements were calculated using the coefficients of the biological curve of crop moisture requirement ($K_b$) that were defined by the former Research Institute of Irrigation Farming in Bratislava for crops grown under irrigation. The coefficients $K_b$ given in the Czech standard ČSN 75 0434 *Water Requirement for Supplemental Irrigation* were applied. Moisture requirement of a crop over the balance period (decade) was calculated from this equation:

$$V_c = K_b \cdot \sum_{i=1}^{n} Sd_i \qquad (1)$$

where: $V_c$ = moisture requirement of a crop (mm)
$K_b$ = coefficient of the moisture requirement of a crop
$\sum_{i=1}^{n} Sd_i$ = sum of average daily values of saturation deficit for $n$-days

The values of saturation deficit in torr are employed in the functions of the above-mentioned standard ČSN. So conversion coefficient 1.33 was used for the system of units SI – hPa.

A rainfall surplus and/or deficit was determined by comparing the values of instantaneous moisture requirement of the crop with rainfall amount in the decadal balance period.

Cumulative curves of rainfall deficits and surpluses in the period of observation were plotted to define the effect of year and area. The total of rainfall deficits shows the potential requirement for supplemental irrigation, total irrigation amount (mm, $m^3.ha^{-1}$) necessary to compensate moisture requirements of crops fluently.

## RESULTS AND DISCUSSION

Table 1 shows differences in meteorological conditions in the period of observation and in both areas. In comparison with normal values, the average air temperature over the growing season was higher at Doksany in all years except the 1st year of observation (1996), at Žatec over the whole period of observation. But rainfall amounts over the growing season were lower in both areas except 1996. Air temperature was highest in 2000 if the averages of the growing season are compared – it was higher by 1.8°C at Doksany, by 2.6°C at Žatec than the normal. The growing season 2000 was poorest in rainfall of all the years of observation. The rainfall amount

Table 1. Meteorological conditions in the growing seasons 1996–2000

| Year | Average daily air temperatures (°C) | | | | | | | Rainfall amounts (mm) | | | | | | | Average daily saturation deficits (hPa) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4. | 5. | 6. | 7. | 8. | 9. | mean | 4. | 5. | 6. | 7. | 8. | 9. | Σ | 4. | 5. | 6. | 7. | 8. | 9. | mean |
| Doksany | | | | | | | | | | | | | | | | | | | | | |
| N | 8.5 | 13.4 | 16.7 | 18.0 | 17.5 | 13.5 | 14.6 | 32.3 | 54.2 | 55.6 | 59.6 | 64.5 | 40.6 | 306.8 | 3.43 | 4.53 | 5.41 | 6.09 | 5.36 | 3.34 | 4.69 |
| 1996 | 9.2 | 13.1 | 17.0 | 16.6 | 17.5 | 11.4 | 14.1 | 16.4 | 85.7 | 54.7 | 80.2 | 77.7 | 38.0 | 352.7 | 5.26 | 3.39 | 5.52 | 4.63 | 4.91 | 2.72 | 4.40 |
| 1997 | 6.5 | 14.7 | 17.4 | 18.2 | 19.6 | 13.7 | 15.0 | 26.6 | 37.0 | 59.6 | 111.2 | 23.9 | 15.8 | 274.1 | 3.16 | 5.98 | 6.53 | 5.58 | 6.85 | 4.38 | 5.41 |
| 1998 | 11.0 | 15.5 | 18.6 | 18.4 | 18.4 | 13.6 | 15.9 | 14.4 | 11.2 | 117.0 | 41.4 | 36.7 | 77.0 | 297.7 | 4.60 | 6.97 | 6.53 | 6.27 | 8.07 | 2.67 | 5.85 |
| 1999 | 10.2 | 15.1 | 16.6 | 20.2 | 18.0 | 17.2 | 16.2 | 12.8 | 18.8 | 58.2 | 46.5 | 30.9 | 43.1 | 210.3 | 4.20 | 6.69 | 5.29 | 7.89 | 7.63 | 5.46 | 6.19 |
| 2000 | 10.8 | 15.4 | 18.8 | 17.8 | 20.4 | 15.2 | 16.4 | 6.2 | 63.3 | 27.7 | 42.0 | 23.6 | 14.3 | 177.1 | 4.52 | 6.22 | 8.03 | 5.81 | 9.44 | 4.84 | 6.48 |
| Žatec | | | | | | | | | | | | | | | | | | | | | |
| N | 7.8 | 12.6 | 16.0 | 17.5 | 16.7 | 13.0 | 13.9 | 29.0 | 59.0 | 56.0 | 71.0 | 55.0 | 34.0 | 304.0 | 3.24 | 4.45 | 5.63 | 5.93 | 5.19 | 3.31 | 4.62 |
| 1996 | 9.2 | 13.5 | 17.2 | 17.0 | 17.6 | 11.5 | 14.3 | 22.1 | 54.3 | 119.0 | 62.1 | 105.5 | 38.3 | 401.3 | 5.14 | 4.39 | 6.94 | 5.68 | 5.32 | 3.09 | 5.09 |
| 1997 | 6.7 | 14.9 | 17.4 | 18.7 | 19.8 | 14.1 | 15.3 | 22.1 | 24.5 | 47.8 | 89.3 | 37.1 | 18.6 | 239.4 | 3.74 | 6.66 | 6.81 | 6.41 | 6.76 | 4.32 | 5.74 |
| 1998 | 10.8 | 15.6 | 18.8 | 18.7 | 18.6 | 13.6 | 16.0 | 15.6 | 9.5 | 72.5 | 57.1 | 39.1 | 62.4 | 256.2 | 5.10 | 7.03 | 7.39 | 6.69 | 7.53 | 2.21 | 5.99 |
| 1999 | 10.0 | 15.3 | 17.0 | 20.8 | 18.1 | 17.3 | 16.4 | 37.8 | 26.4 | 60.1 | 58.2 | 40.3 | 36.1 | 258.9 | 3.95 | 6.56 | 6.13 | 8.83 | 7.67 | 5.36 | 6.42 |
| 2000 | 11.9 | 16.3 | 19.0 | 17.5 | 19.9 | 14.5 | 16.5 | 15.8 | 56.5 | 36.4 | 51.3 | 40.5 | 29.3 | 229.8 | 5.09 | 7.42 | 9.51 | 6.64 | 9.25 | 5.31 | 7.20 |

AVG = arithmetic mean, N = normal values over 1931–1960, and for saturation deficit at Doksany over 1961–1990

Table 2. Moisture deficits and surpluses

| Crop | Locality | Year | Month | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | 4. | 5. | 6. | 7. | 8. | 9. | |
| Winter wheat | Žatec | 1996 | -43 | -26 | +5 | -12 | | | -76 |
| | | 1997 | -22 | -92 | -84 | +4 | | | -194 |
| | | 1998 | -50 | -112 | -69 | -22 | | | -253 |
| | | 1999 | -11 | -98 | -54 | -48 | | | -211 |
| | | 2000 | -49 | -86 | -112 | -15 | | | -262 |
| | Doksany | 1996 | -45 | +22 | -53 | +19 | | | -57 |
| | | 1997 | -9 | -67 | -68 | +38 | | | -106 |
| | | 1998 | -51 | -123 | -4 | -24 | | | -202 |
| | | 1999 | -39 | -107 | -41 | -36 | | | -223 |
| | | 2000 | -53 | -57 | -121 | -20 | | | -251 |
| Sugar beet | Žatec | 1996 | -38 | -5 | +26 | -41 | +15 | -9 | -52 |
| | | 1997 | -20 | -63 | -58 | -25 | -78 | -49 | -293 |
| | | 1998 | -46 | -81 | -43 | -63 | -90 | 28 | -295 |
| | | 1999 | -8 | -60 | -39 | -100 | -85 | -45 | -337 |
| | | 2000 | -45 | -47 | -125 | -68 | -110 | -36 | -431 |
| | Doksany | 1996 | -39 | +40 | -33 | -4 | -5 | -2 | -43 |
| | | 1997 | -8 | -40 | -42 | +12 | -93 | -49 | -220 |
| | | 1998 | -40 | -86 | +10 | -73 | -95 | +38 | -246 |
| | | 1999 | -36 | -68 | -27 | -97 | -94 | -40 | -362 |
| | | 2000 | -49 | -23 | -101 | -62 | -129 | -59 | -423 |
| Early potatoes | Žatec | 1996 | -17 | +7 | +17 | -30 | | | -23 |
| | | 1997 | -12 | -65 | -75 | -37 | | | -189 |
| | | 1998 | -25 | -37 | -29 | -34 | | | -125 |
| | | 1999 | -8 | -77 | -52 | -117 | | | -254 |
| | | 2000 | -27 | -41 | -142 | -80 | | | -290 |
| | Doksany | 1996 | -23 | +38 | -49 | -12 | | | -46 |
| | | 1997 | 0 | -42 | -59 | +2 | | | -99 |
| | | 1998 | -26 | -98 | -8 | -84 | | | -216 |
| | | 1999 | -24 | -85 | -38 | -109 | | | -256 |
| | | 2000 | -36 | -35 | -121 | -73 | | | -265 |
| Hops | Žatec | 1996 | -73 | -19 | +30 | -43 | +8 | | -97 |
| | | 1997 | -47 | -86 | -55 | -27 | -88 | | -303 |
| | | 1998 | -80 | -107 | -38 | -66 | -96 | | -387 |
| | | 1999 | -34 | -75 | -39 | -100 | -102 | | -350 |
| | | 2000 | -79 | -58 | -126 | -67 | -133 | | -463 |
| | Doksany | 1996 | -73 | +28 | -29 | -5 | -10 | | -89 |
| | | 1997 | -31 | -61 | -39 | +9 | -103 | | -225 |
| | | 1998 | -71 | -96 | +11 | -72 | -112 | | -340 |
| | | 1999 | -65 | -84 | -28 | -96 | -110 | | -383 |
| | | 2000 | -79 | -33 | -102 | -62 | -152 | | -428 |

for that growing season was lower than the normal by 74.2 mm at Žatec and even by 129.7 mm at Doksany. Saturation deficit (*Sd*) substantially increased in the evaluated season, mainly at its end. In comparison with the normal, average *Sd* over the growing season 2000 was higher by 1.79 hPa at Doksany and by 2.58 hPa at Žatec.

If the classification scale of normality is used (Klabzuba et al. 1999), growing seasons 1999 and 2000 at Doksany and 1998–2000 at Žatec had extremely above-normal temperatures (extremely warm season), growing seasons 1998 at Doksany and 1997 at Žatec had highly above-normal temperatures (highly warm season). Rainfall amount in the growing season 2000 at Doksany was highly subnormal (highly dry season), in 1999 at Doksany and in 2000 at Žatec subnormal (dry season).

The high frequency of moisture deficit occurrence, i.e. the need of irrigation amounts, was demonstrated in the growing seasons of all 5 years of observation in all evaluated crops in the irrigation areas Poohří and the lower Elbe. Moisture surpluses were scarce, most frequently in 1996. Table 2 documents that total moisture deficits in 1997–2000 were either close to the total moisture requirement defined in the Czech standard ČSN 75 0434 or they were higher. They were highest at Žatec in 2000, when they exceeded the moisture requirement by 32 mm in winter wheat, by 61 mm in sugar beet, by 90 mm in early potatoes and even by 113 mm in hops. Figure 1 shows the distribution of moisture surpluses and defi-

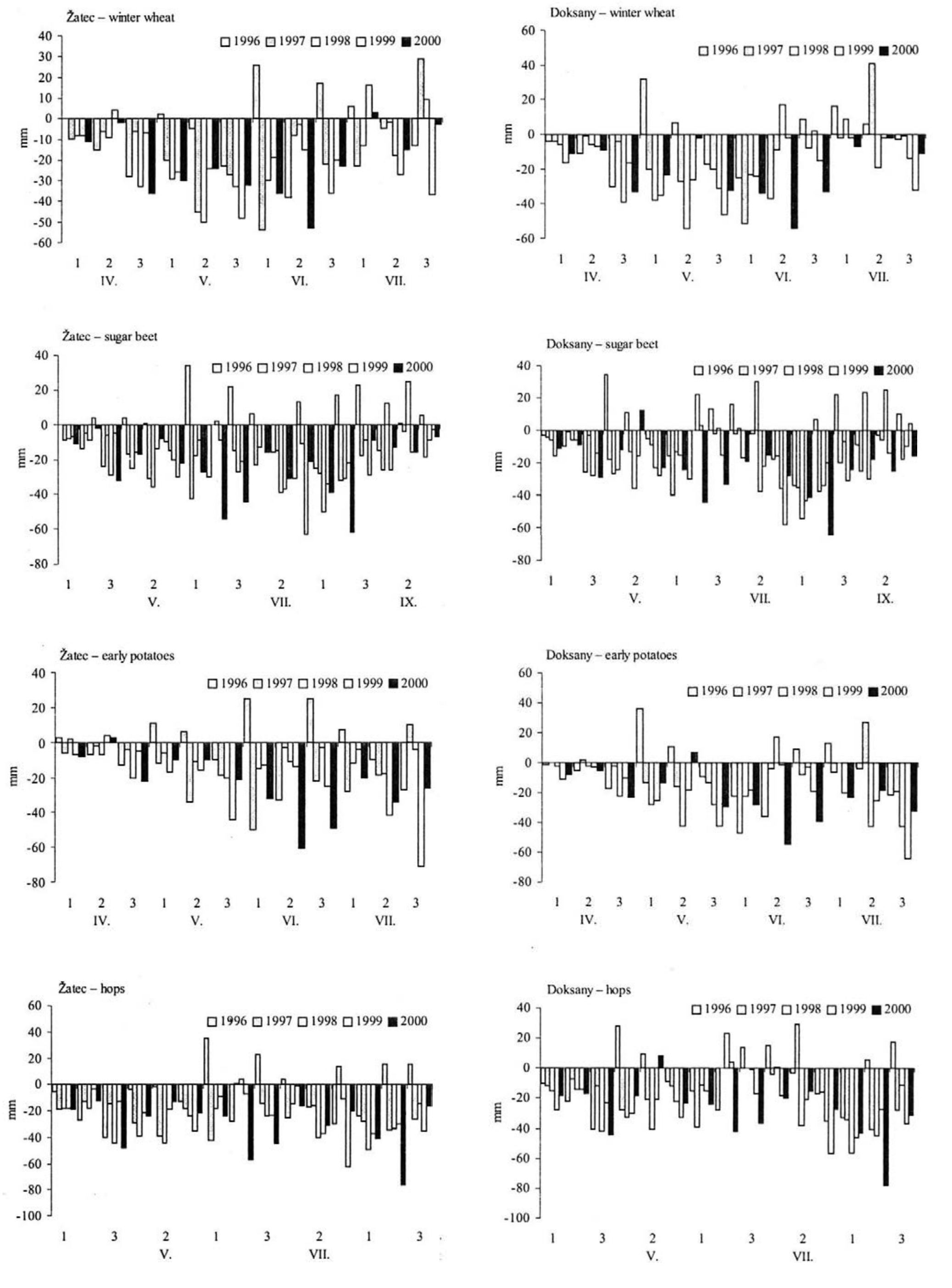


Figure 1. Moisture surpluses and deficits for the areas and crops in 1996–2000 (1–3 decade, I.–XII. month)

cits of the particular crops in the years of observation by the two areas.

Variable conditions of rainfall supply to crops during the growing season (i.e. drought occurrence) are represented by summation curves of moisture deficits and surpluses. Summation curves for the particular crops and areas are shown in Figure 2. The graphs illustrate dominant effect of the year, contingent occurrence of rainfall

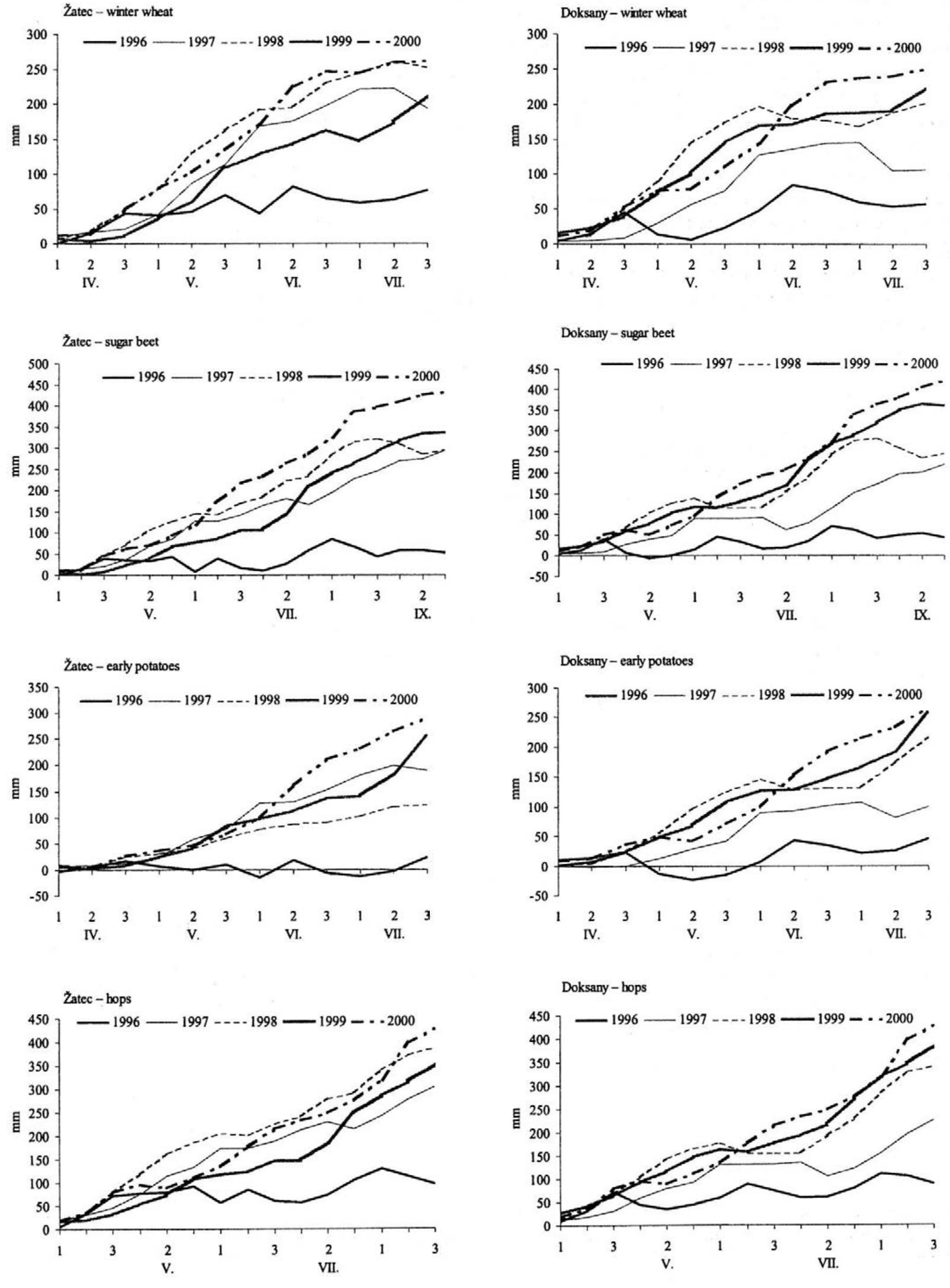


Figure 2. Summation curves of moisture deficits and surpluses of crops in 1996–2000 (1–3 decade, I.–XII. month)

events in daily total. This variability in the compensation of crop moisture requirements is crucial to fix the date of the proven, effective irrigation requirement. Production, yield efficiency of the unit amount of supplied irrigation water ($kg.m^{-3}$) is a decisive criterion to evaluate the effect of irrigation water supply on the elimination of negative impacts of drought on crop yield formation. To achieve the desirable, potential irrigation effect differentiated demands of crops for water supply during their growth and ontogenesis should be respected (the so-called critical moisture period of crops). This is the reason why a regional information system indicating the need of effective irrigation amounts should be introduced, records of the crop irrigation regime balance should be kept for every irrigation area of maximum size 3000 ha and its results should be strictly respected in farming conditions. Otherwise, irrigation will be a little efficient measure to prevent negative drought impacts on agriculture.

## CONCLUSION

The results of the analyses demonstrate the need of supplemental irrigation and its professional exploitation. It is not possible to expect any potential beneficial effects of irrigation water supplies without professional control of the compensation of crop moisture requirements by rainfall.

Irrigation has an irreplaceable regulatory function in the water regime of soils and crops. It is a stabilization element in the production of major field and special crops. It allows a systematic use of the yield potential of soils also in regions with high probability of drought occurrence.

Regions where crop production is evidently dependent on the occurrence of natural rainfall can be considered as areas with high farming risk. These regions are included in the category of less favored areas (LFA), so they require support to the operation of privatized irrigation systems.

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## ABSTRAKT

**Potřeba doplňkových závlah v České republice**

Retrospektivní analýzou byla stanovena úroveň krytí vláhové potřeby brambor, ozimé pšenice, cukrové řepy a chmele dešťovými srážkami ve dvou typických oblastech ČR s vybudovanými a privatizovanými závlahovými soustavami: Poohří (stanice ČHMÚ Žatec) a dolní Labe (stanice ČHMÚ Doksany). Průběh vláhové potřeby sledovaných plodin v období 1996 až 2000 byl vypočten metodou biologické křivky. Bylo zjištěno, že v letech 1997 až 2000 se celkové vláhové deficity buď blížily celkové vláhové potřebě plodin uváděné ČSN 75 0434 *Potřeba vody pro doplňkovou závlahu*, nebo byly i vyšší. Nejvyšší byly v Žatci v roce 2000, kdy překročily vláhovou potřebu ozimé pšenice o 32 mm, cukrové řepy o 61 mm, raných brambor o 90 mm a chmele dokonce o 113 mm.



**Klíčová slova:** sucho; retrospektivní vláhová bilance; doplňkové závlahy



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# The influence of stand establishment and site conditions on grain yield of winter wheat

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**ABSTRACT**

Six year results of study of different ways of winter wheat stand establishment are presented: conventional way = CT, i.e. mouldboard ploughing, seed bed preparation, sowing and protection way = NT, i.e. direct sowing into no-tilled soil by drill machine John Deere 750 with use of surface mulch from post harvest residues of soybean and different levels of nitrogen fertilization (0, 50, 100, 150 $kg.ha^{-1}$) were used in both methods. The field experiments were carried out at three dissimilar sites. The influence of different stand establishment, mulch used and intensity of nitrogen fertilization on grain yield of winter wheat and its structure was analysed. The results of field polyfactorial experiments show possibilities of protection way application of winter wheat stand establishment because grain yields are quite comparable with conventional method. In addition, protection ways are economically favourable in consequence of a saving of working time and direct costs.

**Keywords:** soil tillage; direct drilling; mulch; winter wheat; forecrop soybean; grain yield; catch-crops; nitrogen fertilization

Until quite recently the stands of field crops have been predominantly established with the use of traditional classical methods of soil tillage and sowing. Settled sequences of working operations of conventional soil tillage were applied almost at all sites of our country without great exceptions. Besides, there was a great uniformity of machines used for soil tillage and sowing without regard to the different soil and climatic conditions.

The results of a considerable number of field polyfactorial experiments with various ways of soil tillage in our country and abroad (Maillard et al. 1995, Suškevič 1995, Carefoot and Janzen 1998, Kováč 1998, Mielke and Wilhelm 1999 and others) brought much new knowledge both of influence of minimum tillage on main soil properties and reaction of field crops on reduced soil tillage.

The nature of rational (simplified) ways of stand establishment is founded on utilization of all advantages of minimum and conservation soil tillage to establish a full productive stand with special view of seedbed quality and sowing in optimal term. Use of these technologies results in saving of working time and direct costs. In conservation methods of stand establishment the traditional conception of soil tillage with plowing and classic seed bed preparation gave way to new constructions of farm machinery. It means it does not concern a separated cultivation measures but a new integral technology of stand establishment of field crops.

The aim of this paper is the evaluation of yield response on growing of winter wheat under conservation technology with direct drilling into no tilled soil covered with mulch in comparison with the conventional method.

## MATERIAL AND METHODS

Since 1995 field experiments for observation of impact of different soil tillage have been conducted at three different sites in sugar beet production type. The short characteristics of them are given in Table 1. There was used a split-plot design with four replications in field experiments. The grain and biomass yields were determined on 18 $m^2$ test area at harvest. The field experiments were established as crop rotations and soybean was a forecrop of winter wheat.

### Experimental variants

1. Experimental series (1995–1997)

A) Method of stand establishment

a) taking straw away, plowing to a depth 0.2 m, seed bed preparation, sowing with usual drill machine = CT

b) direct drilling into no-tilled soil with the special drill machine John Deere 750 = NT after:

1) taking straw away (without mulch) = $NT_0$

2) taking straw away, manuring (20 $t.ha^{-1}$) on the soil surface (manure mulch) = $NT_1$

3) leaving straw on the soil surface (straw mulch) = $NT_2$

B) nitrogen fertilization

$N_0 = 0$ $kg.ha^{-1}$

$N_1 = 50$ $kg.ha^{-1}$ (regeneration dose only)

$N_2 = 100$ $kg.ha^{-1}$ (regeneration dose 50%, production one 50%)

2. Experimental series (1997–2000)

A) Method of stand establishment

a) taking straw away, plowing to a depth 0.2 m, seed bed preparation, sowing with usual drill machine = CT

Table 1. Characteristics of the experimental localities

| | Ruzyně | Čáslav | Tišice |
|---|---|---|---|
| Altitude (m) | 350 | 263 | 168 |
| Soil type | Orthic Luvisol | Degraded Luvic Chernozem | Haplic Chernozem |
| Soil texture (topsoil) | clay loam | loam | loamy sand |
| The average annual precipitation (mm) | 545 | 590 | 542 |
| The average annual air temperature (°C) | 8.9 | 8.1 | 8.6 |
| PH (KCl) topsoil | 5.9 | 6.8 | 7.0 |
| P content in topsoil ($mg.kg^{-1}$) | 47 | 63 | 150 |
| K content in topsoil ($mg.kg^{-1}$) | 125 | 139 | 115 |

b) direct drilling into no-tilled soil with the special drill machine John Deere 750 = NT after:

1) taking straw away (without mulch) = $NT_0$
2) leaving straw on the soil surface (straw mulch) = $NT_1$
3) leaving twice as straw amount on the soil surface (double mulch) = $NT_2$

B) nitrogen fertilization

$N_0$ = 50 $kg.ha^{-1}$ (regeneration dose 30, production dose 20 $kg.ha^{-1}$)

$N_1$ = 100 $kg.ha^{-1}$ (regeneration dose 60, production dose 40 $kg.ha^{-1}$)

$N_2$ = 150 $kg.ha^{-1}$ (regeneration dose 60, production dose 90 $kg.ha^{-1}$)

Phosphorus and potassium fertilization was uniform in both series and in all variants in doses P = 24 $kg.ha^{-1}$, K = 83 $kg.ha^{-1}$. The fertilizers were applied on stubble after the harvest of soybean.

Varieties of winter wheat Samanta used in the first series and Mona in the second series were sown with sowing rate 4.5 million seeds per ha. Weeds were controlled according to valid methodologies. Outline of the basic agronomic measures is given in Table 2.

In both experimental series yield of grain and straw and grain yield structure were determined. Signification of yield differences was evaluated by analysis of variance method.

## RESULTS AND DISCUSSION

The grain yield results, which were obtained under different stand establishment and different nitrogen fertilization, are presented in Tables 3–6 including comparison of grain yields between conventional (CT) and direct

Table 2. Basic data on winter wheat stands at experimental sites

| Site/Year | Date of sowing | Date of emergence | Date of harvest | Vegetation period days |
|---|---|---|---|---|
| | | Ruzyně | | |
| 1995 | 6. 10. 1994 | 30. 10. 1994 | 7. 8. 1995 | 305 |
| 1996 | 16. 10. 1995 | 27. 10. 1995 | 20. 8. 1996 | 309 |
| 1997 | 25. 10. 1996 | 3. 11. 1996 | 6. 8. 1997 | 286 |
| 1998 | 6. 10. 1997 | 17. 10. 1997 | 5. 8. 1998 | 304 |
| 1999 | 20. 10. 1998 | 1. 11. 1998 | 3. 8. 1999 | 288 |
| 2000 | 21. 10. 1999 | 3. 11. 1999 | 1. 8. 2000 | 285 |
| | | Čáslav | | |
| 1995 | 12. 10. 1994 | 5. 11. 1994 | 3. 8. 1995 | 296 |
| 1996 | 19. 10. 1995 | 5. 11. 1995 | 13. 8. 1996 | 299 |
| 1997 | 24. 10. 1996 | 31. 10. 1996 | 12. 8. 1997 | 293 |
| 1998 | 14. 10. 1997 | 5. 11. 1997 | 27. 7. 1998 | 287 |
| 1999 | 22. 10. 1998 | 7. 11. 1998 | 3. 8. 1999 | 286 |
| 2000 | 19. 10. 1999 | 2. 11. 1999 | 2. 8. 2000 | 288 |
| | | Tišice | | |
| 1995 | 12. 10. 1994 | 31. 10. 1994 | 26. 7. 1995 | 288 |
| 1996 | 18. 10. 1995 | 8. 11. 1995 | 8. 8. 1996 | 295 |
| 1997 | 24. 10. 1996 | 6. 11. 1996 | 5. 8. 1997 | 286 |
| 1998 | 14. 10. 1997 | 20. 11. 1997 | 21. 7. 1998 | 281 |
| 1999 | 21. 10. 1998 | 10. 11. 1998 | 23. 7. 1999 | 276 |
| 2000 | 19. 10. 1999 | 5. 11. 1999 | 27. 7. 2000 | 291 |

drilling into no-tilled soil (NT). The average results obtained during six-year duration of field experiments show the grain yields of winter wheat on luvisol at Ruzyně site under conventional tillage was achieved at level 5.09 $t.ha^{-1}$ and under no tillage in average of different mulch at level 4.84 $t.ha^{-1}$, which is 5% less than in CT. On degraded chernozem at Čáslav site the relations between both ways of stand establishment were similar, to be more precise in NT grain yield it was 0.82 $t.ha^{-1}$ (13%) lower than in CT. On haplic chernozem (light sand soil) at Tišice site it was the opposite way because grain yield of winter wheat without irrigation was 0.38 $t.ha^{-1}$ (8%) and under irrigation almost 2% higher in NT than in CT.

Comparing grain yields of winter wheat in the best variant of CT and the best one of NT in average of all experimental years, we come to conclusion that in Ruzyně there are no statistically significant differences between observed methods of stand establishment (only 1% for the benefit of CT). In Čáslav the same comparison showed by 4% higher yield of grain in CT than in NT. In Tišice on light soils there were achieved statistically significant differences between the highest grain yield in NT and CT. The average yield of grain in NT was 9% higher without irrigation and 5% higher under irrigation than in CT.

These results confirmed that winter wheat stand establishment by direct drilling into no-tilled soil, especially after one-year legumes with use of their straw and other post harvest residues for mulch is not only quite comparable with conventional method but at localities with light soils this way is more favourable from standpoint of grain production and economy.

This knowledge quite corresponds with conclusions of Procházková and Dovrtěl (2000) or Kováč (1998), who achieved comparable grain yields of winter wheat grown after pea under no-tillage technology as under traditional method in similar field experiments in rather dry conditions of maize production type.

The results also showed the distinct influence of mulch on grain yield of winter wheat at experimental sites. Comparing grain yield from the variant $NT_0$ (without mulch) and $NT_2$ (straw mulch), we found higher yields of grain by influence of mulch applied in Ruzyně by 9.5% (statistically significant), in Čáslav by 5.0% and in Tišice (no irrigation) by 2.6%.

Study of impact of gradated up nitrogen fertilization in both ways of winter wheat stand establishment (Figure 1 and 2) in the first series at Ruzyně and Čáslav site showed statistically significant yield differences among individual levels of nitrogen fertilization, especially in NT variant, more often between the first and second dose of nitrogen. But there was an obvious yield depression in NT variant under all levels of nitrogen fertilization in comparison with CT. It also means that possibility of compensation of absent tillage by application of higher doses of nitrogen was not displayed as a consequence of low efficiency of nitrogen fertilization. At Tišice site on light sand soils there were registered significantly higher yield differences caused by gradated up doses of nitrogen in both ways of tillage both without irrigation and under irrigation.

In the second experimental series in Ruzyně and Čáslav nitrogen fertilization in three determined doses caused the similar trend as in the first series. In Čáslav there were lower yields of grain in NT in comparison with CT under all levels of nitrogen fertilization. On the light soils in Tišice without and under irrigation increase in grain yield caused by gradated up N-doses was not so much considerable in both ways of soil tillage, but in general there is a clear tendency of higher grain yields in NT.

On the basis of presented results, it is possible to say that drilling of winter wheat after good forecrops into no-tilled soil does not call for increase of nitrogen fertilization to achieve the yield level in conventional way of stand establishment (Suškevič 1995, Angelini et al. 1997). But it is necessary to observe mineral N content in the soil during spring period of vegetation and the state of nutrients in plants of winter wheat and on the basis of data obtained to regulate the intensity of nitrogen fertilization. Overview of grain yield structure of winter wheat is put in Table 7.

From the above-mentioned level of individual yield elements it is evident that grain yield of winter wheat in studied ways of stand establishment was formed by random combination among ear number per area, grain number per ear and thousand grain weight. Data in Table 7 lead to conclusion that it is possible to achieve grain yield higher than 6 $t.ha^{-1}$ in the stand the density of which is expressed by starting value 500 $ears.m^{-2}$, which is influenceable especially by way of stand establishment. Our experiments showed that it is possible to achieve this result with the use of sowing winter wheat into no-tilled soil. It was also confirmed in another home experiments (Hrubý 1989).

The effect of increasing intensity of nitrogen fertilization on yield elements formation in both ways of soil tillage at Ruzyně site was quite poor. The highest increase of ear number per area or grain number per ear by use of the highest dose of nitrogen in comparison with the lowest one was only 12%. In Čáslav there was found the impact of increasing intensity of nitrogen fertilization on increase of ear number per area by 14% and grain number per ear by 23% regardless of the way of stand establishment. At Tišice site the effect of increasing doses of nitrogen on yield elements was considerable. Especially ear number per area increased by 32–44% in the first series and by 23–39% in the second series. Number of grain per ear increased by 31% in the first series (0–100 $kg.ha^{-1}$). But in the second series increase of grain number per ear was found neither in CT, nor in NT because these values were the highest under the first dose of nitrogen, i.e. 50 $kg.ha^{-1}$.

The above-mentioned results of polyfactorial field experiments show possibilities of the use of the so-called simplified way of stand establishment of winter wheat in frame of innovated growing technologies for cereals, especially after good forecrops (Miština 1992). Protec-

Table 3. Grain yield of winter wheat at Ruzyně site

| Variant | | | 1995 | 1996 | 1997 | $\bar{x}$ 1995 –1997 | 1998 | 1999 | 2000 | $\bar{x}$ 1998 –2000 | $\bar{x}$ 1995 –2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conventional tillage | | $N_0$ | 5.86 | 5.20 | 4.23 | **5.10** | – | – | – | – | – |
| | | $N_1$ | 5.78 | 5.85 | 4.68 | **5.44** | 4.50 | 5.10 | 3.67 | **4.42** | **4.93** |
| | | $N_2$ | 5.78 | 5.99 | 4.93 | **5.57** | 5.56 | 5.50 | 3.74 | **4.93** | **5.25** |
| | | $N_3$ | – | – | – | – | 6.27 | 5.55 | 3.59 | **5.14** | – |
| | | $\bar{x}$ | **5.81** | **5.68** | **4.61** | **5.37** | **5.44** | **5.38** | **3.67** | **4.83** | **5.09** |
| Conservation tillage | $NT_0$ | $N_0$ | 4.96 | 2.74 | 4.57 | **4.09** | – | – | – | – | – |
| | | $N_1$ | 4.93 | 3.79 | 4.68 | **4.47** | 4.34 | 4.56 | 4.11 | **4.34** | **4.41** |
| | | $N_2$ | 4.73 | 4.63 | 5.37 | **4.91** | 4.36 | 5.33 | 4.32 | **4.67** | **4.79** |
| | | $N_3$ | – | – | – | – | 5.03 | 5.73 | 4.23 | **5.00** | – |
| | | $\bar{x}$ | **4.87** | **3.72** | **4.87** | **4.49** | **4.58** | **5.21** | **4.22** | **4.67** | **4.60** |
| | $NT_1$ | $N_0$ | 4.87 | 3.77 | 4.73 | **4.46** | – | – | – | – | – |
| | | $N_1$ | 4.64 | 4.10 | 5.19 | **4.64** | 4.73 | 5.88 | 4.02 | **4.88** | **4.76** |
| | | $N_2$ | 4.70 | 4.63 | 5.45 | **4.93** | 4.93 | 6.23 | 4.18 | **5.11** | **5.02** |
| | | $N_3$ | – | – | – | – | 5.13 | 6.14 | 3.95 | **5.07** | – |
| | | $\bar{x}$ | **4.74** | **4.17** | **5.12** | **4.68** | **4.93** | **6.08** | **4.05** | **5.02** | **4.89** |
| | $NT_2$ | $N_0$ | 5.54 | 4.03 | 4.63 | **4.73** | – | – | – | – | – |
| | | $N_1$ | 5.61 | 4.74 | 5.14 | **5.16** | 4.22 | 5.54 | 3.95 | **4.57** | **4.87** |
| | | $N_2$ | 5.34 | 5.96 | 5.01 | **5.44** | 4.58 | 6.33 | 3.98 | **4.96** | **5.20** |
| | | $N_3$ | – | – | – | – | 4.63 | 6.28 | 4.00 | **4.97** | – |
| | | $\bar{x}$ | **5.50** | **4.91** | **4.93** | **5.11** | **4.48** | **6.05** | **3.98** | **4.83** | **5.04** |
| | NT | $\bar{x}$ | **5.04** | **4.27** | **4.97** | **4.76** | **4.66** | **5.78** | **4.08** | **4.84** | **4.84** |

$DT_{0.05} = 0.31$, $DT_{0.01} = 0.43$

Table 4. Grain yield of winter wheat at Čáslav site

| Variant | | | 1995 | 1996 | 1997 | $\bar{x}$ 1995 –1997 | 1998 | 1999 | 2000 | $\bar{x}$ 1998 –2000 | $\bar{x}$ 1995 –2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conventional tillage | | $N_0$ | 6.67 | 6.88 | 6.30 | **6.62** | – | – | – | – | – |
| | | $N_1$ | 7.47 | 6.86 | 6.22 | **6.85** | 5.04 | 6.95 | 6.16 | **6.05** | **6.45** |
| | | $N_2$ | 6.84 | 6.63 | 5.88 | **6.45** | 5.03 | 7.16 | 7.08 | **6.42** | **6.44** |
| | | $N_3$ | – | – | – | – | 5.72 | 7.44 | 7.09 | **6.75** | – |
| | | $\bar{x}$ | **6.99** | **6.79** | **6.13** | **6.64** | **5.26** | **7.18** | **6.78** | **6.52** | **6.44** |
| Conservation tillage | $NT_0$ | $N_0$ | 4.96 | 4.96 | 3.19 | **4.37** | – | – | – | – | – |
| | | $N_1$ | 6.12 | 5.75 | 4.95 | **5.61** | 4.49 | 4.10 | 6.01 | **4.87** | **5.24** |
| | | $N_2$ | 6.38 | 6.64 | 4.38 | **5.80** | 4.39 | 4.57 | 7.04 | **5.33** | **5.57** |
| | | $N_3$ | – | – | – | – | 4.62 | 5.00 | 7.12 | **5.58** | – |
| | | $\bar{x}$ | **5.82** | **5.78** | **4.17** | **5.26** | **4.50** | **4.56** | **6.72** | **5.26** | **5.40** |
| | $NT_1$ | $N_0$ | 5.40 | 4.68 | 4.09 | **4.72** | – | – | – | – | – |
| | | $N_1$ | 6.66 | 5.80 | 4.47 | **5.64** | 4.41 | 5.22 | 5.71 | **5.11** | **5.38** |
| | | $N_2$ | 7.50 | 6.45 | 5.78 | **6.58** | 4.57 | 6.00 | 6.71 | **5.76** | **6.17** |
| | | $N_3$ | – | – | – | – | 4.64 | 5.87 | 6.97 | **5.83** | – |
| | | $\bar{x}$ | **6.52** | **5.64** | **4.78** | **5.65** | **4.54** | **5.70** | **6.46** | **5.57** | **5.78** |
| | $NT_2$ | $N_0$ | 6.02 | 3.88 | 3.61 | **4.50** | – | – | – | – | – |
| | | $N_1$ | 6.44 | 5.67 | 5.28 | **5.80** | 3.93 | 4.70 | 5.85 | **4.83** | **5.32** |
| | | $N_2$ | 7.66 | 6.35 | 5.41 | **6.47** | 4.62 | 5.43 | 6.63 | **5.56** | **6.02** |
| | | $N_3$ | – | – | – | – | 4.38 | 5.61 | 6.89 | **5.63** | – |
| | | $\bar{x}$ | **6.71** | **5.30** | **4.77** | **5.59** | **4.31** | **5.25** | **6.46** | **5.34** | **5.67** |
| | NT | $\bar{x}$ | **6.35** | **5.57** | **4.57** | **5.50** | **4.45** | **5.17** | **6.55** | **5.39** | **5.62** |

$DT_{0.05} = 0.33$, $DT_{0.01} = 0.47$

Table 5. Grain yield of winter wheat at Tišice site (no irrigation)

| Variant | | | 1995 | 1996 | 1997 | $\bar{x}$ 1995 –1997 | 1998 | 1999 | 2000 | $\bar{x}$ 1998 –2000 | $\bar{x}$ 1995 –2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conventional tillage | | $N_0$ | 2.47 | 3.12 | 3.66 | **3.08** | – | – | – | – | – |
| | | $N_1$ | 3.93 | 4.48 | 4.92 | **4.44** | 3.05 | 5.31 | 3.55 | **3.97** | **4.21** |
| | | $N_2$ | 5.69 | 5.43 | 5.45 | **5.52** | 3.09 | 5.66 | 4.52 | **4.42** | **4.97** |
| | | $N_3$ | – | – | – | – | 3.22 | 6.15 | 4.89 | **4.75** | – |
| | | $\bar{x}$ | **4.03** | **4.34** | **4.68** | **4.35** | **3.12** | **5.71** | **4.32** | **4.38** | **4.59** |
| Conservation tillage | $NT_0$ | $N_0$ | 2.69 | 2.64 | 3.31 | **2.88** | – | – | – | – | – |
| | | $N_1$ | 4.78 | 4.30 | 4.71 | **4.60** | 3.33 | 5.67 | 4.25 | **4.42** | **4.51** |
| | | $N_2$ | 5.60 | 5.19 | 5.38 | **5.39** | 3.58 | 6.84 | 4.68 | **5.03** | **5.21** |
| | | $N_3$ | – | – | – | – | 3.34 | 6.89 | 5.10 | **5.11** | – |
| | | $\bar{x}$ | **4.36** | **4.04** | **4.47** | **4.29** | **3.42** | **6.47** | **4.68** | **4.85** | **4.86** |
| | $NT_1$ | $N_0$ | 2.47 | 3.10 | 3.68 | **3.08** | – | – | – | – | – |
| | | $N_1$ | 4.57 | 4.58 | 5.09 | **4.75** | 3.66 | 5.68 | 4.73 | **4.69** | **4.72** |
| | | $N_2$ | 5.75 | 5.65 | 5.38 | **5.59** | 4.08 | 6.36 | 5.30 | **5.25** | **5.42** |
| | | $N_3$ | – | – | – | – | 3.57 | 6.80 | 5.40 | **5.26** | – |
| | | $\bar{x}$ | **4.26** | **4.44** | **4.72** | **4.47** | **3.77** | **6.28** | **5.14** | **5.07** | **5.07** |
| | $NT_2$ | $N_0$ | 3.42 | 2.63 | 5.53 | **3.86** | – | – | – | – | – |
| | | $N_1$ | 4.73 | 3.85 | 5.11 | **4.56** | 3.29 | 5.88 | 5.35 | **4.84** | **4.70** |
| | | $N_2$ | 5.53 | 5.32 | 5.68 | **5.51** | 3.24 | 6.54 | 5.27 | **5.02** | **5.27** |
| | | $N_3$ | – | – | – | – | 3.58 | 6.74 | 5.68 | **5.33** | – |
| | | $\bar{x}$ | **4.56** | **3.93** | **5.44** | **4.64** | **3.37** | **6.39** | **5.43** | **5.06** | **4.98** |
| | NT | $\bar{x}$ | **4.39** | **4.14** | **4.88** | **4.47** | **3.52** | **6.38** | **5.08** | **4.99** | **4.97** |

$DT_{0.05}$ = 0.40, $DT_{0.01}$ = 0.57

Table 6. Grain yield of winter wheat at Tišice site (irrigation)

| Variant | | | 1995 | 1996 | 1997 | $\bar{x}$ 1995 –1997 | 1998 | 1999 | 2000 | $\bar{x}$ 1998 –2000 | $\bar{x}$ 1995 –2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conventional tillage | | $N_0$ | 2.70 | 3.12 | 4.71 | **3.51** | – | – | – | – | – |
| | | $N_1$ | 4.74 | 4.81 | 5.86 | **5.14** | 3.76 | 6.04 | 5.13 | **4.98** | **5.06** |
| | | $N_2$ | 5.56 | 5.76 | 7.66 | **6.33** | 4.37 | 6.24 | 5.95 | **5.52** | **5.93** |
| | | $N_3$ | – | – | – | – | 4.63 | 7.40 | 6.25 | **6.09** | – |
| | | $\bar{x}$ | **4.33** | **4.56** | **6.08** | **4.99** | **4.25** | **6.56** | **5.78** | **5.53** | **5.49** |
| Conservation tillage | $NT_0$ | $N_0$ | 2.55 | 2.74 | 3.66 | **2.98** | – | – | – | – | – |
| | | $N_1$ | 4.33 | 4.59 | 5.26 | **4.73** | 4.22 | 5.69 | 5.74 | **5.22** | **4.98** |
| | | $N_2$ | 5.95 | 5.21 | 6.38 | **5.85** | 4.79 | 6.97 | 6.56 | **6.11** | **5.98** |
| | | $N_3$ | – | – | – | – | 4.70 | 6.75 | 6.66 | **6.04** | – |
| | | $\bar{x}$ | **4.28** | **4.18** | **5.10** | **4.52** | **4.57** | **6.47** | **6.32** | **5.79** | **5.48** |
| | $NT_1$ | $N_0$ | 2.19 | 3.17 | 4.49 | **3.28** | – | – | – | – | – |
| | | $N_1$ | 4.57 | 4.93 | 5.59 | **5.03** | 4.82 | 6.22 | 6.15 | **5.73** | **5.38** |
| | | $N_2$ | 5.86 | 5.59 | 6.60 | **6.02** | 5.23 | 7.46 | 6.56 | **6.42** | **6.22** |
| | | $N_3$ | – | – | – | – | 5.51 | 7.58 | 7.09 | **6.73** | – |
| | | $\bar{x}$ | **4.21** | **4.56** | **5.56** | **4.78** | **5.19** | **7.09** | **6.60** | **6.29** | **5.80** |
| | $NT_2$ | $N_0$ | 2.50 | 2.63 | 3.40 | **2.84** | – | – | – | – | – |
| | | $N_1$ | 4.46 | 4.47 | 5.08 | **4.67** | 4.00 | 6.41 | 5.95 | **5.45** | **5.06** |
| | | $N_2$ | 5.86 | 5.34 | 5.82 | **5.67** | 4.65 | 7.44 | 6.51 | **6.20** | **5.94** |
| | | $N_3$ | – | – | – | – | 4.51 | 7.66 | 6.83 | **6.33** | – |
| | | $\bar{x}$ | **4.27** | **4.15** | **4.77** | **4.40** | **4.39** | **7.17** | **6.43** | **5.99** | **5.50** |
| | NT | $\bar{x}$ | **4.25** | **4.30** | **5.14** | **4.57** | **4.72** | **6.91** | **6.45** | **6.02** | **5.59** |

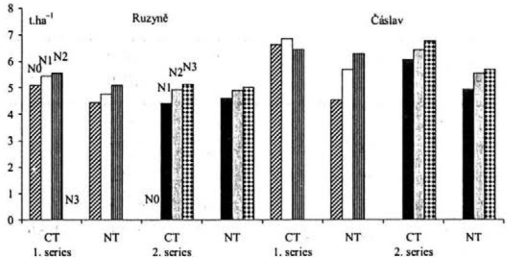


Figure 1. The influence of nitrogen fertilization on grain yield of winter wheat on average of experimental years at individual sites

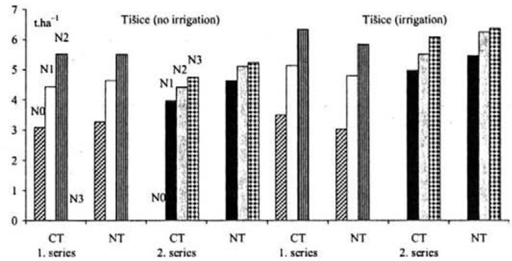


Figure 2. The influence of nitrogen fertilization on grain yield of winter wheat on average of experimental years at Tišice site

Table 7. Grain yield structure of winter wheat (the average data)

| Variant | | 1st experimental sequence | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H |
| | | Ruzyně | | | | | | | |
| CT | $N_0$ | 5.10 | 512 | 21 | 47.3 | – | – | – | – |
| | $N_1$ | 5.44 | 530 | 21 | 48.6 | 4.42 | 408 | 20 | 53.4 |
| | $N_2$ | 5.57 | 515 | 22 | 48.7 | 4.93 | 410 | 23 | 53.1 |
| | $N_3$ | – | – | – | – | 5.14 | 441 | 22 | 52.9 |
| $NT_0$ | $N_0$ | 4.09 | 378 | 23 | 47.2 | – | – | – | – |
| | $N_1$ | 4.47 | 413 | 23 | 47.3 | 4.34 | 331 | 25 | 53.3 |
| | $N_2$ | 4.91 | 423 | 24 | 47.5 | 4.67 | 334 | 26 | 53.9 |
| | $N_3$ | – | – | – | – | 5.00 | 334 | 28 | 54.1 |
| $NT_1$ | $N_0$ | 4.46 | 419 | 23 | 46.9 | – | – | – | – |
| | $N_1$ | 4.64 | 410 | 24 | 47.1 | 4.88 | 337 | 27 | 53.1 |
| | $N_2$ | 4.93 | 431 | 24 | 47.3 | 5.11 | 362 | 26 | 53.9 |
| | $N_3$ | – | – | – | – | 5.07 | 354 | 27 | 53.9 |
| $NT_2$ | $N_0$ | 4.73 | 378 | 27 | 46.4 | – | – | – | – |
| | $N_1$ | 5.16 | 423 | 26 | 46.3 | 4.57 | 336 | 25 | 53.5 |
| | $N_2$ | 5.44 | 394 | 29 | 47.6 | 4.96 | 352 | 26 | 53.8 |
| | $N_3$ | – | – | – | – | 4.97 | 373 | 25 | 53.9 |
| | | Čáslav | | | | | | | |
| CT | $N_0$ | 6.62 | 522 | 29 | 43.9 | – | – | – | – |
| | $N_1$ | 6.85 | 519 | 31 | 42.8 | 6.05 | 421 | 27 | 52.8 |
| | $N_2$ | 6.45 | 534 | 28 | 43.5 | 6.42 | 435 | 28 | 52.9 |
| | $N_3$ | – | – | – | – | 6.75 | 492 | 26 | 53.7 |
| $NT_0$ | $N_0$ | 4.37 | 569 | 17 | 43.9 | – | – | – | – |
| | $N_1$ | 5.61 | 584 | 22 | 44.4 | 4.87 | 365 | 24 | 56.1 |
| | $N_2$ | 5.80 | 620 | 21 | 44.0 | 5.33 | 373 | 26 | 55.9 |
| | $N_3$ | – | – | – | – | 5.58 | 409 | 24 | 56.0 |
| $NT_1$ | $N_0$ | 4.72 | 566 | 19 | 44.6 | – | – | – | – |
| | $N_1$ | 5.64 | 603 | 21 | 44.2 | 5.11 | 374 | 24 | 56.3 |
| | $N_2$ | 6.58 | 630 | 24 | 44.3 | 5.76 | 421 | 24 | 55.9 |
| | $N_3$ | – | – | – | – | 5.83 | 405 | 25 | 56.7 |
| $NT_2$ | $N_0$ | 4.50 | 545 | 19 | 44.1 | – | – | – | – |
| | $N_1$ | 5.80 | 556 | 24 | 44.0 | 4.83 | 350 | 24 | 57.2 |
| | $N_2$ | 6.47 | 611 | 24 | 43.5 | 5.56 | 401 | 25 | 56.0 |
| | $N_3$ | – | – | – | – | 5.63 | 400 | 26 | 55.0 |

| Variant | | 2nd experimental sequence | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H |
| | | Tišice (no irrigation) | | | | | | | |
| CT | $N_0$ | 3.08 | 435 | 17 | 42.7 | – | – | – | – |
| | $N_1$ | 4.44 | 546 | 18 | 44.3 | 3.97 | 391 | 21 | 47.3 |
| | $N_2$ | 5.52 | 613 | 20 | 45.3 | 4.42 | 475 | 19 | 47.8 |
| | $N_3$ | – | – | – | – | 4.75 | 545 | 18 | 49.5 |
| $NT_0$ | $N_0$ | 2.88 | 428 | 16 | 42.7 | – | – | – | – |
| | $N_1$ | 4.60 | 497 | 21 | 44.2 | 4.42 | 401 | 24 | 45.9 |
| | $N_2$ | 5.39 | 565 | 21 | 45.6 | 5.03 | 506 | 21 | 46.4 |
| | $N_3$ | – | – | – | – | 5.11 | 513 | 22 | 46.2 |
| $NT_1$ | $N_0$ | 3.08 | 397 | 18 | 43.0 | – | – | – | – |
| | $N_1$ | 4.75 | 493 | 22 | 44.4 | 4.69 | 429 | 23 | 48.2 |
| | $N_2$ | 5.59 | 538 | 23 | 45.5 | 5.25 | 470 | 24 | 47.5 |
| | $N_3$ | – | – | – | – | 5.26 | 528 | 20 | 48.7 |
| $NT_2$ | $N_0$ | 3.86 | 420 | 21 | 43.3 | – | – | – | – |
| | $N_1$ | 4.56 | 509 | 20 | 44.8 | 4.84 | 418 | 25 | 46.5 |
| | $N_2$ | 5.51 | 559 | 22 | 45.8 | 5.02 | 461 | 23 | 48.2 |
| | $N_3$ | – | – | – | – | 5.33 | 515 | 22 | 47.6 |
| | | Tišice (irrigation) | | | | | | | |
| CT | $N_0$ | 3.51 | 477 | 18 | 43.4 | – | – | – | – |
| | $N_1$ | 5.14 | 554 | 21 | 45.0 | 4.98 | 407 | 25 | 49.3 |
| | $N_2$ | 6.33 | 641 | 22 | 45.9 | 5.52 | 487 | 23 | 49.6 |
| | $N_3$ | – | – | – | – | 6.09 | 554 | 22 | 50.0 |
| $NT_0$ | $N_0$ | 2.98 | 445 | 16 | 42.1 | – | – | – | – |
| | $N_1$ | 4.73 | 554 | 19 | 44.0 | 5.22 | 461 | 23 | 50.0 |
| | $N_2$ | 5.85 | 629 | 21 | 44.8 | 6.11 | 536 | 22 | 51.4 |
| | $N_3$ | – | – | – | – | 6.04 | 569 | 20 | 52.1 |
| $NT_1$ | $N_0$ | 3.28 | 448 | 18 | 41.1 | – | – | – | – |
| | $N_1$ | 5.03 | 572 | 20 | 43.9 | 5.73 | 462 | 24 | 51.6 |
| | $N_2$ | 6.02 | 633 | 21 | 44.5 | 6.42 | 524 | 24 | 51.3 |
| | $N_3$ | – | – | – | – | 6.73 | 585 | 22 | 52.9 |
| $NT_2$ | $N_0$ | 2.84 | 444 | 15 | 42.0 | – | – | – | – |
| | $N_1$ | 4.67 | 588 | 18 | 43.4 | 5.45 | 462 | 23 | 50.7 |
| | $N_2$ | 5.67 | 626 | 20 | 44.7 | 6.20 | 522 | 23 | 51.5 |
| | $N_3$ | – | – | – | – | 6.33 | 616 | 20 | 52.2 |

A = grain yield ($t.ha^{-1}$), B = ear number per $m^2$, C = grain number per ear (calculated), D = thousand grain weight (g), E = grain yield ($t.ha^{-1}$), F = ear number per $m^2$, G = grain number per ear (calculated), H = thousand grain weight (g) tion stand establishment of winter wheat with the use of straw mulch of soybean is also economically favourable because in comparison with conventional method it is possible to achieve up to 80% saving of working time (i.e. 1.75 $hr.ha^{-1}$) and approximately 1000 CZK savings of direct costs per ha (Javůrek 2000).

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## ABSTRAKT

### Vliv způsobu založení porostu a podmínek stanoviště na výnos ozimé pšenice

V polyfaktoriálních pokusech na třech různých stanovištích (Praha-Ruzyně, Čáslav a Tišice u Všetat) byl během šestiletého období sledován vliv různého založení porostu ozimé pšenice po předplodině sóji na výši a strukturu výnosu zrna. V první pokusné sérii (1995–1997) byl porovnáván konvenční způsob (CT) založení porostu ozimé pšenice (orba, předseťová příprava a setí) s ochranným způsobem (NT), který spočíval ve výsevu ozimé pšenice do nezpracované půdy secím strojem John Deere 750 a obsahoval subvarianty: $NT_1$ – bez mulče (sláma sóji po sklizni odklizena); $NT_2$ – mulč chlévským hnojem po úklidu slámy (rozmetání 20 $t.ha^{-1}$ na povrch pole); $NT_3$ – mulč slámou sóji. V obou způsobech založení porostu bylo uplatněno stupňované hnojení dusíkem v dávkách: $N_0 = 0$, $N_1 = 50$, $N_2 = 100$ $kg.ha^{-1}$. V druhé pokusné sérii (1998–2000) byl způsob založení porostu ozimé pšenice stejný, avšak na subvariantě $NT_2$ byla ponechána sláma sóji na poli jako mulč a v $NT_3$ bylo použito dvojnásobné množství sójové slámy jako mulč. Hnojení dusíkem bylo opět v obou variantách stejné : $N_1 = 50$, $N_2 = 100$, $N_3 = 150$ $kg.ha^{-1}$. Výnosy zrna ozimé pšenice (tab. 3–6), založené konvenčním způsobem (CT), činily v průměru šesti let na stanovišti v Ruzyni 5.09 $t.ha^{-1}$, po přímém výsevu do mulče (NT) byly nižší o 5%, v Čáslavi na CT dosáhly 6.44 $t.ha^{-1}$, na NT byly nižší o 13 %. V Tišicích byly výnosy zrna ozimé pšenice naopak vyšší na NT, a to bez závlahy o 8 %, v závlaze o 2 % oproti CT. Použitý mulč na NT vykazoval na všech stanovištích kladný vliv na výnosy zrna ozimé pšenice. Byly zjištěny vyšší výnosy zrna ozimé pšenice na subvariantě s mulčem oproti subvariantě bez mulče – v Ruzyni o 9,5 %, v Čáslavi o 5 % a v Tišicích bez závlahy o 2,6 %. Stupňované dávky dusíku u sledovaných způsobů zakládání porostu ovlivnily výnosy zrna ozimé pšenice (obr. 1 a 2) tak, že v 1. pokusné sérii na stanovištích v Ruzyni a v Čáslavi byly zjištěny významné rozdíly zejména v NT, a to častěji mezi první a druhou dávkou dusíku. V Tišicích, na lehčích půdách mělo stupňování dávek dusíku za následek vysoce významné zvyšování výnosu zrna ozimé pšenice (o 35–45 %) bez ohledu na způsob založení porostu. Ve 2. pokusné sérii v Ruzyni a v Čáslavi měly stupňované dávky dusíku obdobný vliv na výnosy zrna pouze s tím rozdílem, že v Čáslavi na variantě NT byly výnosy nižší než na CT. V Tišicích se stupňováním dávek dusíku výnosy zrna ozimé pšenice zvyšovaly, avšak přírůstky již nedosahovaly takové výše, jako v 1. pokusné sérii, zejména u nejvyšší dávky dusíku. Analýzu struktury výnosů zrna ozimé pšenice jak ve vztahu ke sledovaným způsobům zakládání porostů, tak i k použitému hnojení dusíkem uvádí tab. 7. Výsledky šestiletého polyfaktoriálního polního pokusu celkově prokazují možnost uplatnění ochranného způsobu zakládání porostu ozimé pšenice.

**Klíčová slova:** zpracování půdy; přímý výsev; mulč; ozimá pšenice; předplodina sója; výnos zrna; meziplodiny; hnojení dusíkem

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# Z VĚDECKÉHO ŽIVOTA

## O průběhu 19. Evropské regionální konference Mezinárodní komise pro závlahy a odvodňování (ICID)

*Brněnské Kongresové centrum hostilo na začátku června delegáty 19. Evropské regionální konference Mezinárodní komise pro závlahy a odvodňování (ICID), která se po 31 letech vrátila opět na území ČR. Pod záštitou ministra zemědělství ji uspořádal Český výbor ICID. Vedle hlavní konference se za podpory FAO (Organizace pro výživu a zemědělství OSN) uskutečnily v Praze dva workshopy, věnované závlahám a odvodnění zemědělských půd v postkomunistických zemích střední a východní Evropy.*
*Všechna jednání, různorodá co do početnosti auditoria i projednávaných otázek, měla společné hlavní téma „Udržitelné využívání půdy a vody". Celkem se na jednáních objevilo na 200 účastníků z 27 zemí a zaznělo více než 70 ústních příspěvků, které jsou k dispozici na CD-ROM vydaném u příležitosti konference. Proběhla rovněž prezentace 40 posterů.*

V postkomunistických zemích střední a východní Evropy, ve kterých byly v minulosti závlahy a odvodňování podporovány státem (a to většinou bez ohledu na vlastnické vztahy a ochranu životního prostředí), zájem o tato zařízení dnes značně poklesl. Někde probíhá jejich privatizace, jinde se stát stále ještě angažuje. Většinou se ale využívá jen zlomek vybudovaných závlahových soustav, zanedbává se jejich údržba a rekonstrukce. Vliv těchto zařízení na životní prostředí (ať kladný, nebo záporný) není veřejnosti dostatečně znám.

Přitom závlahy i odvodňování patří ke kulturní krajině a mají i v nových ekonomických podmínkách nezanedbatelný význam. Sehrávají svou roli při sociálním rozvoji venkova a údržbě krajiny. Jsou neoddělitelnou složkou komplexního hospodaření s vodními zdroji, jak je chápe legislativa EU (zejména Rámcová vodohospodářská směrnice).

Konference se shodla na tom, že jsou nutné rozsáhlé technologické inovace založené na multidisciplinárním přístupu a mezinárodní spolupráci. Odvodňovací technologie totiž mohou být dobře využity k řízené revitalizaci vodních toků a mokřadů.

Delegáti přiznali, že finanční podpora státu provozovatelům a uživatelům závlahových a odvodňovacích soustav v té míře, jako tomu bylo v minulosti, je nemyslitelná. Vynaložené náklady se zákonitě musejí navracet, tzn. že je musejí vynakládat zejména ti, kteří z odvodňování a závlah přímo nebo nepřímo profitují. V přechodném období, kdy je v některých zemích soukromý zemědělský sektor slabý a bez podpory by nepřežil, je však žádoucí pomoc státu a nadnárodních institucí.

Značná pozornost byla věnována způsobům předvídání a zmírňování následků extrémních hydrologických jevů, jako jsou povodně, sucha a eroze půdy. Konference zdůraznila potřebu více se věnovat bezprostředním praktickým otázkám, jako je spolupráce s pojišťovnami a obecné zvyšování připravenosti ke zvládání extrémních jevů. Globální změny klimatu činí tyto otázky ještě aktuálnějšími.

Delegáti dále zdůraznili, že v EU byla již přijata řada směrnic k ochraně kvantity a kvality vodních zdrojů. K zajištění jejich účinnosti je nutná soustava analytických a manažerských nástrojů, založených na měření a modelování složitých procesů. Některé takové nástroje a jejich aplikace byly na konferenci předvedeny. Ochrana vod a půd je však složitá a drahá záležitost a je nutno nebát se hledat nekonvenční řešení.

V závěru konference, která se stejně jako workshopy k naší upřímné radosti setkala u odborné i laické veřejnosti s velmi příznivým ohlasem, byla předjednána řada námětů na budoucí společné projekty, přičemž hlavními tématy byly komplexní management oblastí ovlivněných povrchovou těžbou nerostů, rekonstrukce a modernizace závlahových soustav, informační a vzdělávací zabezpečení aplikace směrnic EU a optimální využití odvodňovacích soustav ve vodním hospodářství malých povodí.

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