Transfer of the hop (*Humulus lupulus* L.) alpha-bitter acid content to progenies of F_1 and I_1 generations in selected parental components

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ABSTRACT

In the period 1999–2001 the transfer of α-acid content from selected parents to their progenies was evaluated. Four female plants (English varieties Target and Yeoman, German variety Magnum and Czech variety Premiant) and four male plants from the gene resources of male hops (82/6, 86/4, 87/3, clone 72) were chosen as the initial material. Progenies of F_1 generation of Magnum and Yeoman show significantly higher α-acid content compared to the progenies of other female hops. Progenies of F_1 generation of male plants 86/4 and 87/3 show significantly higher α-acid content compared to the progenies of other male plants. Progenies of I_1 generation of Magnum variety and male 86/4 contain the highest amount of α-acids. Progenies of F_1 generation have higher α-acid content at the 99% probability level compared to the progenies of I_1 generation. Progenies of both generations show nearly the same variability.

Keywords: hops; *Humulus lupulus* L.; progenies; F_1 generation; I_1 generation; α-bitter acids

Hop breeding is a very complex process. Currently it is aimed at the crossing of suitable parental components. Problems of hop breeding can be summarised in the following points: hop is a dioecious plant, it is highly heterozygous and promising plants require planting for a long time (Nesvadba 2001). Hop is one of not many cultural dioecious plants. Hop is cultivated for its generative organs – hop cones on female plants (Neve 1991). Hop cones are produced by female plants only, male plants enter into the crossing process like an unknown pollinator (Beránek 1997). Therefore it is necessary to test female plants for the transfer of required traits to progenies (Nesvadba et al. 1999).

Hop resins, hop oils and other secondary metabolites formed in cones are the main hop components from the aspect of brewing technology (Peacock 1998). α-Bitter acids are the most important component of hop resins. During wort boiling they isomerise into iso-α-acids, main carriers of beer bitter taste (Hughes and Simpson 1996). The content of α-bitter acids is based on the activity of many genes. Therefore parental plants have to be tested for their ability to transfer this trait to progenies and simultaneously for mutual combining capacity. It can be supposed that the progenies of mothers with high α-acid content will increase the content of this component. Determination of the amount of α-bitter acids in male plants is very difficult due to their very low content. Male plants are tested for their ability to transfer α-bitter acids to progenies before hybridisation.

This paper describes in detail procedures of testing the transfer of α-acids to progenies of F_1 generation. The abilities of male and female plants to transfer the above-mentioned trait provide important information for the whole hop breeding process. Progenies of I_1 generation were evaluated simultaneously. Transfer of traits from original parents within the framework of inbreeding is perceptible. Information what generation is the most suitable for selection for required traits should be obtained. Methods for evaluation of differences between the parental progenies are also described.

MATERIAL AND METHODS

The evaluation of model hybridisation was performed in the period 1999–2001. At first parental components to obtain F_1 progenies were chosen. In the collection of hop varieties in Hop Research Institute Co., Ltd., in Žatec there are almost 300 various genotypes from which the following mother plants were selected:

Target – English variety, content of α-bitter acids is in the range of 9–13% w/w.
Yeoman – English variety, content of α-bitter acids is in the range of 10–14% w/w.
Magnum – German variety, content of α-bitter acids is in the range of 12–16% w/w.
Premiant – Czech variety, content of α-bitter acids is in the range of 7–11% w/w.

The content of α-bitter acids was obtained from several years evaluation of hops cultivated in variety tests

Table 1. Statistical characteristics of α-bitter acid content (% w/w) in the progenies of F_1 generation

Progeny	Origin mother (P_1)	×	father (P_2)	Arithmetic mean (x)	Standard deviation (s)	Relative standard deviation (RSD%)	Index
H 25	Target	×	82/6	6.46	1.667	25.80	0.911
H 26	Target	×	86/4	5.22	1.766	33.83	0.736
H 27	Target	×	87/3	5.69	2.549	44.80	0.803
H 28	Target	×	clone 72	5.31	1.175	22.13	0.749
H 29	Magnum	×	82/6	7.77	1.703	21.91	1.096
H 30	Magnum	×	86/4	8.42	1.939	23.03	1.188
H 31	Magnum	×	87/3	7.85	2.154	27.43	1.107
H 32	Magnum	×	clone 72	7.06	1.667	23.62	0.996
H 33	Yeoman	×	82/6	7.18	1.850	25.77	1.013
H 34	Yeoman	×	86/4	8.55	1.695	19.78	1.206
H 35	Yeoman	×	87/3	8.76	1.706	19.45	1.236
H 36	Yeoman	×	clone 72	5.69	1.831	32.18	0.803
H 37	Premiant	×	82/6	5.68	0.785	13.83	0.801
H 38	Premiant	×	86/4	6.22	1.713	27.53	0.877
H 39	Premiant	×	87/3	5.58	1.134	20.32	0.787
H 40	Premiant	×	clone 72	5.37	1.285	23.92	0.757
Collection of progenies				7.09	2.113	29.79	1.000

and gene collection. Male plants were selected in a nursery of male cultivars where almost 110 genotypes are cultivated. The chosen male plants of different genetic origin were as follows:

82/6 – hybrid genotype from Bor variety (mother component), Saaz variety accounts for a 24% proportion in the origin.

86/4 – hybrid genotype from Sládek variety (mother component), Saaz variety accounts for a 33% proportion in the origin.

87/3 – hybrid genotype from Premiant variety (mother component), Saaz variety accounts for a 50% proportion in the origin.

Osvald's clone 72 – male plant, found in a hop yard planted with Osvald's clone 72 in Smilovice (Žatec region). It is the case of an originally female plant turning into a male one.

Selected male and female plants were mutually crossed within the framework of model hybridisation (diallel hybridisation). Progenies of F_1 generation were obtained in this way. Progenies of I_1 generation were obtained by crossing daughter × son in F_1 generation of selected female (Target, Magnum, Yeoman, Premiant) and male (82/6, 86/4, 87/3, clone 72) plants. Unfortunately, not all progenies of male Osvald's clone 72 were obtained. Therefore the results of this plant are not included in the evaluation of I_1 generation.

The content of α-bitter acids was determined by conductometric titration according to a method defined by the standard ČSN 46 2520-15. The content of α-bitter acids is expressed as a lead conductance value (LCV) and based on dry matter in weight percentage (% w/w). Basic statistical parameters were used for statistical evaluation: arithmetic mean (x), standard deviation (s) and relative standard deviation (RSD); t-test, which evaluates all

Table 2. Mean values of α-acid content (LCV) in the progenies of F_1 generation

Female / Male	Target	Magnum	Yeoman	Premiant	***x***	*s*	*RSD* (%)	Succession
82/6	6.46	7.77	7.18	5.68	**7.17**	1.772	24.71	3
86/4	5.22	8.42	8.55	6.22	**7.51**	2.295	30.56	1
87/3	5.69	7.85	8.76	5.58	**7.45**	2.400	32.21	2
clone 72	5.31	7.06	5.69	5.37	**6.25**	1.668	26.69	4
x	**6.08**	**8.19**	**8.01**	**6.08**	**7.09**			
s	1.935	1.959	2.189	1.186	**2.113**			
RSD (%)	31.83	23.92	27.33	19.15	**29.79**			
Succession	3	1	2	4				

Table 3. Statistical significance of differences in α-acid content determined by *t*-test at various levels of probability

	Magnum				**86/4**		
Yeoman	–	**Yeoman**		**87/3**	–	**87/3**	
Target	α = 0.01	α = 0.01	**Target**	**82/6**	α = 0.02	α = 0.1	**82/6**
Premiant	α = 0.01	α = 0.01	–	**clone 72**	α = 0.01	α = 0.01	α = 0.05

individuals from progenies, was employed to determine differences between the progenies of tested male and female plants; pair *t*-test (Meloun and Militký 1994) was used for the assessment of traits between average values of progenies of F_1 generation and I_1 generation. Distinctness of groups was determined on the basis of significance level α, which expresses probability of distinctness of the tested groups ($P = 1 - \alpha$).

The frequency of individuals with determined level of the evaluated trait was investigated in the obtained progenies. The identity of distribution was used for the evaluation of individual sets with different number of individuals. Two methods were applied to determine differences between the progenies of tested female and male plants: χ^2-test and Kolmogorov-Smirnov test for two independent sets. Kolmogorov-Smirnov test (*K-S*-test) was used for the evaluation of distribution identity. Linear regression was used to evaluate the influence of the expression of traits in parents and their transfer to the studied progenies.

RESULTS AND DISCUSSION

Transfer of α-bitter acid content to the progenies of F_1 generation

Average content of α-bitter acids (LCV) in the progenies of F_1 generation was 7.09% w/w (Table 1). Average content of α-bitter acids above the level of 8.0% was

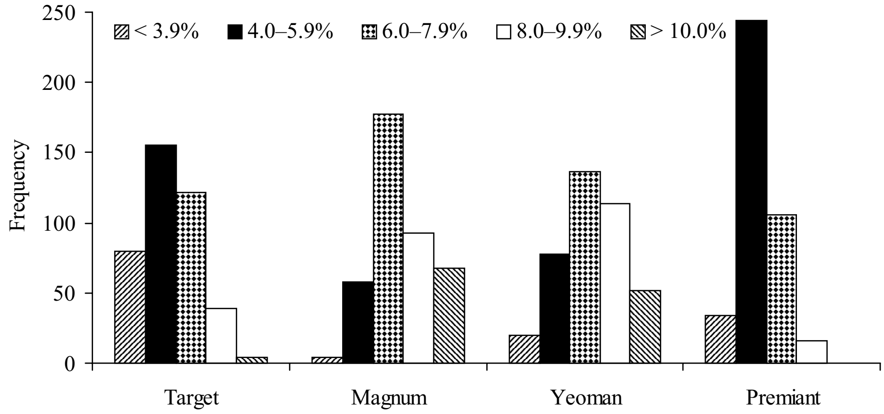

Figure 1. Distribution of progenies of F_1 generation in classification categories according to α-acid content for female plants

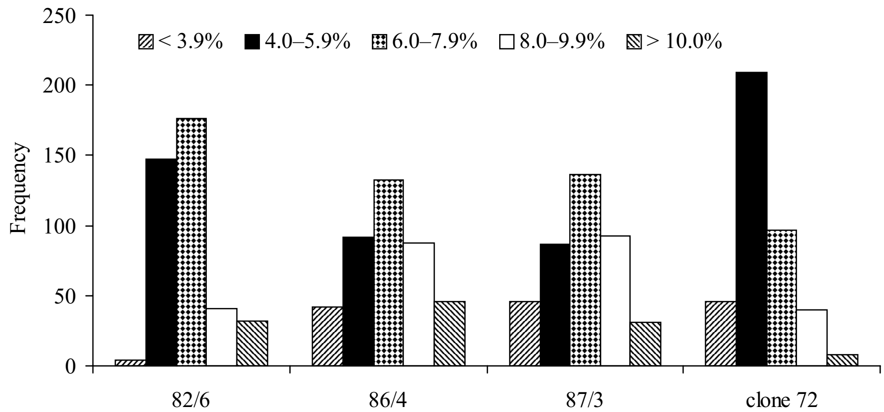

Figure 2. Distribution of progenies of F_1 generation in classification categories according to α-acid content for male plants

Table 4. Statistical significance of differences in the number distribution at a probability level by non-parametric tests

	Magnum				82/6		
Yeoman	–* –**	**Yeoman**		**87/3**	–* –**	**87/3**	
Target	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	**Target**	**86/4**	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	**86/4**
Premiant	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	**clone 72**	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**

* Kolmogorov-Smirnov test, ** χ^2-test

found in progenies H 35 (8.76%), H 34 (8.55%) and H 30 (8.42%). The lowest average content of α-bitter acids was determined in progenies H 26 (5.22%), H 28 (5.31%) and H 40 (5.37%). The variability expressed by *RSD* was in the range between 13.83% (H 37) and 44.80% (H 27). The parents Yeoman × 86/4 and Yeoman × 87/3 had a good combining ability. Their progenies had the highest average content of α-bitter acids and the lowest value of *RSD* (below 20%).

The data in Table 2 show that the progenies of varieties Magnum (8.19%) and Yeoman (8.01%) have the highest mean value of α-acids in F1 generation, progenies of Target and Premiant have the same mean 6.08%. The progenies of Target variety show the highest variability in α-acids content (*RSD* = 31.83%), the lowest variability was found in the progenies of Premiant variety. The progenies of male plants 82/6, 86/4 and 87/3 have an average α-acid content above the level of 7% w/w, progenies of male Osvald's clone 72 reach the mean value 6.25%.

Statistical significance of the mean values of α-acid content for the individual progenies was determined by *t*-test (Table 3). The progenies of Magnum and Yeoman varieties have a significantly higher α-acid content at 99% probability level compared to Target and Premiant ones. Statistical significance of differences between the other progenies was not determined. The progenies of male plants 86/4 and 87/3 have a statistically significantly higher α-acid content compared to the other ones. The progenies of male plant 82/6 have a statistically significant higher α-acid content compared to clone 72 progenies at 95% probability level.

Differences between the progenies of parental components were evaluated by the distribution of total number of individuals into classification categories. The progenies of Target variety have the highest number in the category of 4.0–5.9% α-acid content (Figure 1). Progenies of Magnum and Yeoman varieties have the highest number in the category of 6.0–7.9% α-acid content. Premiant progenies have the highest number in the category of 4.0–5.9% α-acid content, no individual with α-acid content above 10% w/w was found.

The progenies of male plants 82/6, 86/4 and 87/3 have the highest number of individuals in the category of 6.0–7.6% α-acid content (Figure 2). Progenies of male plants 86/4 and 87/3 show practically identical distribu-

Table 5. Statistical characteristics of α-acid content (% w/w) in the progenies of I_1 generation (index of progeny mean/progeny set)

Progeny (H) $P_1 \times P_2$	Parent from F_1 generation daughter		son	Arithmetic mean (x)	Standard deviation (s)	Relative standard deviation (*RSD*%)	Index
H 30 (Target × 82/6)	H 25	×	H 25	6.30	1.582	25.11	1.027
H 31 (Target × 86/4)	H 26	×	H 26	5.29	1.705	32.24	0.861
H 32 (Target × 87/3)	H 27	×	H 27	3.12	1.492	47.77	0.509
H 34 (Magnum × 82/6)	H 29	×	H 29	6.22	1.851	29.77	1.013
H 35 (Magnum × 86/4)	H 30	×	H 30	7.01	1.772	25.27	1.142
H 36 (Magnum × 87/3)	H 31	×	H 31	5.82	0.805	13.83	0.948
H 37 (Yeoman × 82/6)	H 33	×	H 33	3.70	1.265	34.24	0.602
H 38 (Yeoman × 86/4)	H 34	×	H 34	5.65	1.570	27.77	0.920
H 39 (Yeoman × 87/3)	H 35	×	H 35	7.17	1.731	24.15	1.167
H 41 (Premiant × 82/6)	H 37	×	H 37	5.50	0.798	14.52	0.896
H 42 (Premiant × 86/4)	H 38	×	H 38	6.71	1.352	20.16	1.092
H 45 (Premiant × 87/3)	H 39	×	H 39	5.68	1.374	24.19	0.925
Collection of progenies				6.14	1.876	30.52	1.000

Table 6. Mean values of α-acid content (LCV) in the progenies of I_1 generation according to parental pairs

Female Male	Target	Magnum	Yeoman	Premiant	*x*	*s*	*RSD* (%)	Succession
82/6	6.30	6.22	3.70	5.50	**5.85**	1.786	30.51	3
86/4	5.29	7.01	5.65	6.71	**6.66**	1.753	26.30	1
87/3	3.12	5.82	7.17	5.68	**5.90**	1.976	33.52	2
x	**5.39**	**6.79**	**5.91**	**6.46**	**6.14**			
s	2.031	1.662	2.072	1.342	**1.876**			
RSD (%)	37.66	24.50	35.08	30.77	**30.52**			
Succession	4	1	3	2				

tion into classification categories. Progenies of male clone 72 have the highest number in the category of 4.0–5.9% α-acid content.

Statistical significance of differences between the progenies was also evaluated by non-parametric tests (Table 4). Statistical significance of differences between Magnum and Yeoman by *K-S*-test and χ^2-tests was not proved. Target and Premiant show a statistically significant difference between the distribution of the number of individuals at 99% probability level in spite of the same mean value of α-acid content (6.08%). Progenies of male plants 86/4 and 87/3 show the same identity of number distribution in non-parametric tests. Statistically significant differences in the distribution of individuals at 99% probability level were determined between the other evaluated progenies.

The transfer of α-acid content trait from mother plants to progenies is almost identical with the values of original varieties. Magnum and Yeoman varieties have the highest α-acid content in the range of 13–15% w/w. Their progenies also have the statistically significantly highest α-acid content. The progenies of Target and Premiant hops show the identical mean value in spite of different α-acid content in mother varieties. Target has α-acid content in the range of 10–13% w/w, Premiant in the range of 7–11% w/w. It means that α-acid content in the progenies of F_1 generation is also influenced by male plants. The progenies of male plants 86/4 and 87/3 show a statistically significantly higher α-acid content.

It can be supposed that the α-acid content in the progenies of F_1 generation will not correspond with the α-acid content in mother plants. Simultaneously, it has to be taken into consideration that the level of α-acid content in progenies will be influenced by male components in which it is difficult to determine the α-acid content correctly. These results correspond with Haunold et al. (1983) findings regarding the influence of male plants on α-acid content in progenies.

Transfer of α-acid content in progenies of I_1 generation

In the framework of hybridisation between the relatives progenies of I_1 generation were obtained. As the hop is a dioecious plant, I_1 generation can be obtained by crossing of F_1 parental components. The aim is to determine the transfer of the trait by inbreeding daughter × son into I_1 generation. The evaluation was limited only to male plants 82/6, 86/4, 87/3 because the progenies of clone 72 were not obtained. It is evident from the above-described procedure that trait transfer will be influenced by the original parental components of F_1 generation as well as by the selected parents (son × daughter) for I_1 generation. Son and daughter selected in F_1 generation represent mean values of progenies by their parameters. The original parental components are as follows: P_1 – female plants Target, Magnum, Yeoman, Premiant; P_2 – male plants 82/6, 86/4, 87/3, clone 72. For example the progenies of H 30 I_1 generation arose as follows:

P_1 (Target)	×	P_2 (82/6)
	↓	
F_1	H 25	
H 25 (daughter)	×	H 25 (son)
	↓	
I_1	H 30	

The highest α-acid content in I_1 generation, summarised in Table 5, was found in progenies H 35 (7.01%)

Table 7. Statistical significance of differences in α-acid content at a probability level in the progenies of parental components

	Magnum					
Premiant	α = 0.01	**Premiant**			**86/4**	
Yeoman	α = 0.01	α = 0.01	**Yeoman**	**87/3**	α = 0.01	**87/3**
Target	α = 0.01	α = 0.01	α = 0.01	**82/6**	α = 0.01	–

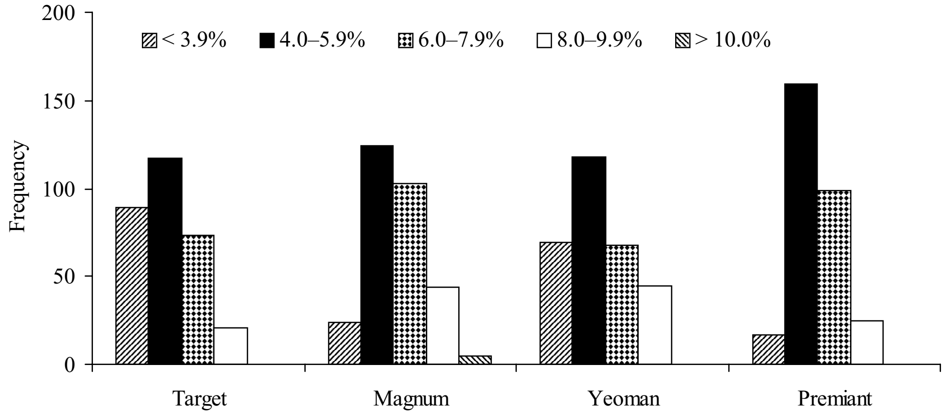

Figure 3. Distribution of progenies of I_1 generation in classification categories according to α-acid content for female plants

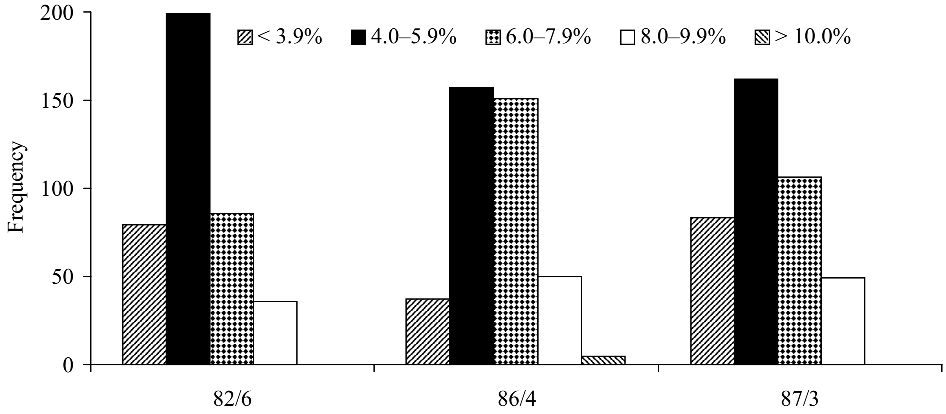

Figure 4. Distribution of progenies of I_1 generation in classification categories according to α-acid content for male plants

and H 39 (7.17%). The lowest variability was recorded in progeny H 36 (*RSD* = 13.83%). Progenies H 32 (3.12%) and H 37 (3.70%) had the lowest α-acid content, progeny H 32 had the highest variability (47.77%) simultaneously.

The highest mean value of α-acid content shown in Table 6 was found in the progenies of Magnum variety (6.79%) which simultaneously had the lowest variability (*RSD* = 24.5%). On the contrary, the progenies of variety Target had the lowest mean α-acid content (5.39%) and the highest variability (*RSD* = 37.66%). The progenies of male plants also show different means of α-acid content. The progenies of male 86/4 had the highest mean value of α-acid content (6.66%) and the lowest level of variability (*RSD* = 26.3%). The progenies of males 82/6 and 87/3 reached practically the identical mean value of α-acid content 5.85 and 5.90%, respectively.

Statistical significance of differences in α-acid content between the evaluated progenies was determined by *t*-test (Table 7). The progenies evaluated according

Table 8. Statistical significance of differences in the number distribution at a probability level by non-parametric tests

	Magnum				86/4	
Premiant	–* α = 0.01**	**Premiant**		**87/3**	α = 0.01* α = 0.01**	**87/3**
Target	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	**Target**	**82/6**	α = 0.01* α = 0.01**	–* α = 0.01**
Yeoman	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**	α = 0.01* α = 0.01**			

* Kolmogorov-Smirnov test, ** χ^2-test

to male plants have statistical significance of differences in α-acid content at 99% probability level. The progenies of male plant 86/4 have a statistically significantly higher content of α-acid content at 99% probability level in comparison with the progenies of other male plants. No statistical significance was found between the progenies of males 82/6 and 87/3.

Differences between the progenies of parental components were evaluated according to the distribution of the number of individuals in classification categories. All progenies have the highest number of individuals in the category of α-acid content in the range of 4.0–5.9% w/w (Figure 3). The highest number of individuals with the content of α-acids below 3.0% was found in the progenies of Target variety. Only a few individuals in the progenies of Magnum variety have the content of α-acids above the level of 10% w/w.

The progenies of male plant 86/4 show practically the same number of individuals in the categories 4.0–5.9 and 6.0–7.9% of α-acid content (Figure 4). The progenies of males 82/6 and 87/3 have a similar distribution of individuals.

Statistical significance of differences between the progenies was also evaluated by non-parametric tests (Table 8). The results of *K-S*-test and χ^2-tests confirm that the progenies of male and female plants show different distribution of the number of individuals. Contrary to *t*-test, *K-S*-test did not prove any difference in the distribution of Magnum and Premiant varieties. *K-S*-test did not prove the different distribution between 87/3 and 82/6 progenies. χ^2-test confirmed different distribution of the number of individuals at 99% probability level in all evaluated progenies.

The progenies of I_1 generation show the lower mean value ($x = 6.14\%$) compared to F_1 generation ($x = 7.09\%$) at 99% probability level. The difference was determined by pair *t*-test. The results show that I_1 progenies originating from inbreeding have almost the same variability ($RSD = 30.52\%$) compared to the progenies of F_1 generation ($RSD = 29.79\%$). It can be stated that selections for α-acid content in the progenies of F_1 generation are more suitable due to statistically significantly higher values of α-acids compared to the progenies of I_1 generation.

CONCLUSIONS

The results show that the most suitable choice of individuals from the viewpoint of α-acid content are selections from the progenies of F_1 generation. Progenies of investigated generations show nearly the same variability. The progenies of F_1 generation show a higher α-acid content compared to I_1 generation. The varieties Magnum and Yeoman confer a higher α-acid content to their progenies from F_1 generation compared to Target and Premiant varieties. The obtained results confirm a significant influence of male plants on α-acid content in the progenies of F_1 generation.

Parametric tests are a suitable tool for the testing of progenies from individual generations. Non-parametric tests show larger differences of results. The results point out problems of α-acid content transfer to progenies. They confirm the need of finding suitable parental components with good combining ability. In the breeding process it is necessary:

1. to test potential parental components;
2. to determine the transfer of the trait of interest (α-acid content) to progenies;
3. to search the most suitable parental components.

Presented results should be considered as particular being related to the evaluated parental components.

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ABSTRAKT

Přenos obsahu alfa-hořkých kyselin u chmele (*Humulus lupulus* L.) na potomstva F_1 a I_1 generace u vybraných rodičovských komponentů

V letech 1999 až 2001 byl hodnocen přenos obsahu α-hořkých kyselin z vybraných rodičů na potomstva. Jako výchozí materiál byly zvoleny čtyři samičí rostliny (anglické odrůdy Target a Yeoman, německá odrůda Magnum a česká odrůda Premiant) z genetických zdrojů ČR a čtyři samičí rostliny (82/6, 86/4, 87/3 a klon 72) z genofondu samčích rostlin ČR. Potomstva F_1 generace po odrůdách Magnum a Yeoman mají průkazně vyšší obsah α-hořkých kyselin než potomstva ostatních samičích rostlin. Potomstva F_1 generace po samčích rostlinách 86/4 a 87/3 mají průkazně vyšší obsah α-hořkých kyselin než potomstva ostatních samčích rostlin. Potomstva I_1 generace po odrůdě Magnum a samčí rostlině 86/4 opět mají nejvyšší obsah α-hořkých kyselin. V celkovém hodnocení potomstva F_1 generace mají s 99% pravděpodobností vyšší obsah α-hořkých kyselin než potomstva I_1 generace. Potomstva obou generací vykazují téměř shodnou variabilitu.

Klíčová slova: chmel; *Humulus lupulus* L.; potomstva; F_1 generace; I_1 generace; α-hořké kyseliny

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