POTASSIUM AND PROLINE CONTENT IN HOP LEAVES AS BIOCHEMICAL MARKER FOR DROUGHT STRESS TOLERANCE

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ABSTRACT

In the investigation biochemical markers which could serve as drought stress tolerance pointers were measured. Potassium and proline content in hop leaves of different hop varieties (four Slovenian, two German and one South African hop varieties, wild hop labelled JUG2 and hybrid 279/122) at different water regimes were observed. The investigation showed biochemical markers that will contribute to faster selection of drought stress tolerant hybrids and the method of testing. Hop plants had higher potassium content in leaves under the drought stress conditions compared to adequate water supply, but the values differed from variety to variety. From July to August proline content in leaves decreased by more than 80 % when plants were watered regularly while it increased in the leaves of plants that were exposed to water stress. Preliminary results can be a basis for further investigations since no clear boundary was detected among the varieties.

Key words: hop, *Humulus lupulus* L., potassium content, proline content, drought stress

VSEBNOST KALIJA IN PROLINA V LISTIH HMELJA KOT BIOKEMIČNIH MARKERJEV TOLERANTNOSTI NA SUŠNI STRES

IZVLEČEK

V raziskavi smo preučevali biokemične markerje, ki bi lahko služili kot pokazatelji odpornosti oziroma občutljivosti hmelja na sušni stres. Analizirali smo vsebnost kalija in prolina v listih različnih sort hmelja pri različnih vodnih režimih. Vključene bo bile štiri slovenske sorte hmelja, dve nemški, ena južnoafriška, divji hmelj z oznako JUG2 in križanec 279/122. Raziskava je nakazala biokemične markerje, ki bi lahko pripomogli k hitrejši selekciji na sušo bolj tolerantnih križancev in razvoju metode testiranja. Hmelj je imel namreč večjo vsebnost kalija v listih v razmerah sušnega stresa v primerjavi z ustrezno preskrbo z vodo, vendar so bile vrednosti različne glede na sorto. Od julija do avgusta se je vsebnost prolina v listih zmanjšala za več kot 80 % pri redno zalivanih rastlinah, medtem ko se je v listih rastlin, izpostavljenih sušnemu stresu, povečala. Predstavljeni preliminarni rezultati služijo kot osnova za nadaljnje raziskave, saj niso bile nakazane jasne razlike med sortami.

Ključne besede: hmelj, *Humulus lupulus* L., vsebnost kalija, vsebnost prolina, sušni stres

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1 INTRODUCTION

Hop presents an important export material for Slovenia as about 95 % of the yield is sold on the global market. In the changing climatic conditions with frequent drought it is important to plant drough tolerant varieties which give yields with solid quality in the years with different water supply.

Environmental stresses present the most limiting factors in agricultural productivity. Apart from biotic stress caused by plant pathogens, there are a number of abiotic stresses such as extreme temperatures, drought, salinity, heavy metals and radiation which all have detrimental effects on plant growth and yield. However, certain plant species and ecotypes have developed various mechanisms to adapt to such stress conditions. For sustainable agriculture development, future crops should have abiotic stress resistant traits and a mechanism for stress tolerance.

Many plants accumulate organic osmolytes in response to the imposition of environmental stresses that cause cellular dehydration. Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selection of drought-resistant varieties of different species [1,2,4,5,6]. Potassium uptake as an index for screening cultivars on drought resistance was also reported [10].

Harmful effect of drought stress can also be alleviated with potassium fertilization. In plant cells potassium has an affect on osmosis and probably changes pH of the stroma in chloroplast. Increased potassium content in cells increases their osmotic potential, which leads to increased acceptance of water from the companion cells and to changes of turgor [9].

Despite the fact that each hop producing country has its own breeding program, so far tolerance to abiotic stress as a main breeding goal has not been included to such an extent as tolerances to biotic stresses (diseases, pests). Some studies in the field of hop plant response to unpleasant climatic conditions have been reported lately [3,12]. It was agreed previously that the depth of active roots and the leaf mass quantity are of extreme importance under drought conditions [7]. The concentration of cell sap, the stomas response and net photosynthesis have already been partly studied in physiological studies [11].

Tolerance of Slovenian hop varieties to drought has mainly been established from practical experiences. Considerable influence of weather conditions on the growth and yield of Savinjski golding variety has been recorded [9], while Atlas variety has shown quite good drought tolerance also in extreme years (own observations). Aurora variety, which is widely grown in Slovenian hop gardens, is well known for its plasticity under different environmental conditions.

Biochemical markers which could serve as drought stress tolerance pointers were determined in the research. Potassium and proline contents were analyzed in the leaves of different hop varieties under different water regimes.

2 MATERIAL AND METHODS

2.1 Material

Hop cultivars included in the research were:
- four Slovenian hop varieties: Aurora, Celeia, Cicero, Dana,
- two German varieties: Hallertauer Merkur, Hallertauer Taurus,
- one South African variety: Southern Star,
- wild hop labelled JUG2 and
- perspective hybrid 279/122.

The varieties were divided into two categories based on preliminary investigations: more tolerant to drought stress (Aurora, Celeia, Southern Star, JUG2) and more sensitive to drought stress (Hallertauer Merkur, Cicero).

2.2 Field and pot trials

<u>Field trial</u>
Five plants of each included hop variety were planted in the field in 2004. They were cultivated according to the good agricultural practice and exposed to natural weather conditions.

<u>Pot trial</u>
Pot trial was conducted in a glasshouse in 2004. Five pots (plants) of each variety listed above were included in the trial. Pots were outside during winter and displaced into the glasshouse in spring. Three different treatments were carried out:
- drought stress simulation (DS): in the middle of July plants were watered for the last time and left without water for a month,
- plants were watered regularly (WR) and
- plants were watered by natural rainfall (WP).

2.3 Sampling

Samples were taken in 2005 and 2006. Hop plant leaves were sampled at three different heights of the plant – lower, middle and upper part (each in the same quantity) until one litre sample was collected. Leaves were sampled in the middle of July (when the plants were last watered at DS treatment – 18 July), in the middle of August (18 August) and in October of 2005. In 2006 sampling was performed based on the experiences from the previous season: in mid-July (18 July), beginning of August (3 August) and mid-August (18 August).

2.4 Chemical analyses

In leaf samples moisture content was determined in general, proline content was analyzed spectrofotometricaly (MKH 14; in-house method) while total amount of potassium was determined with MKH 22 method (in-house method).

2.5 Weather conditions

In 2005 season drought stress was not recorded in the field due to favourable weather conditions (Figure 1). Spring was relatively cold and rainy which retarded hop growth. In May temperatures rose and hop plants grew fast. In the middle of May a sudden cooling with a large amount of precipitation occurred. In spite of a short period of warm weather with enough precipitation in June, cold weather which continued throughout July caused slower growth of hop plants. In August relatively cold and wet weather continued which resulted in lower quality of yield.

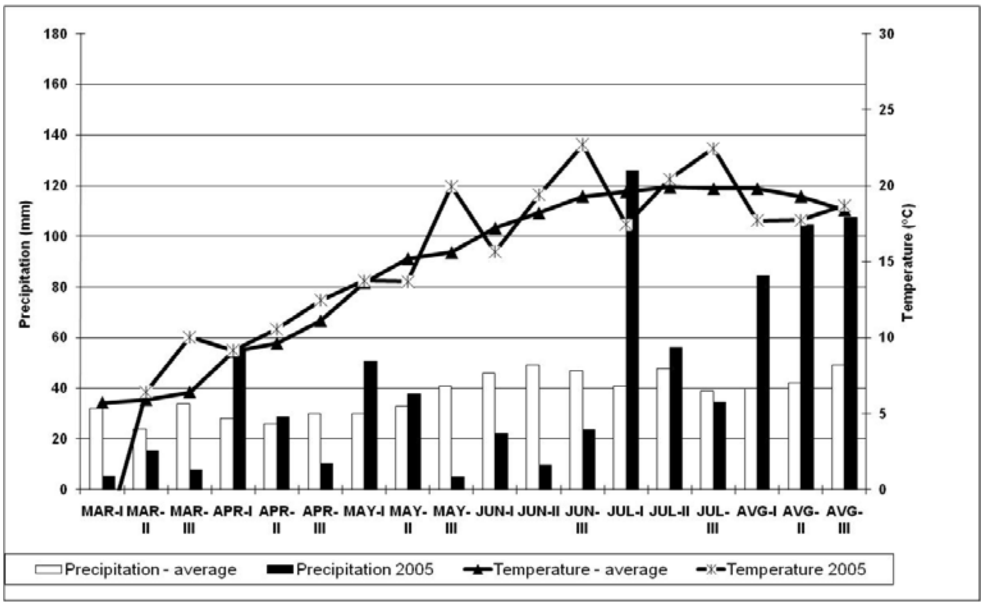

Figure 1: Precipitation amount and average decade's temperatures in growth season of hop in 2005 compared to long term average
Slika 1: Padavine in povprečne dekadne temperature v rastni sezoni hmelja v letu 2005 v primerjavi z dolgoletnim povprečjem

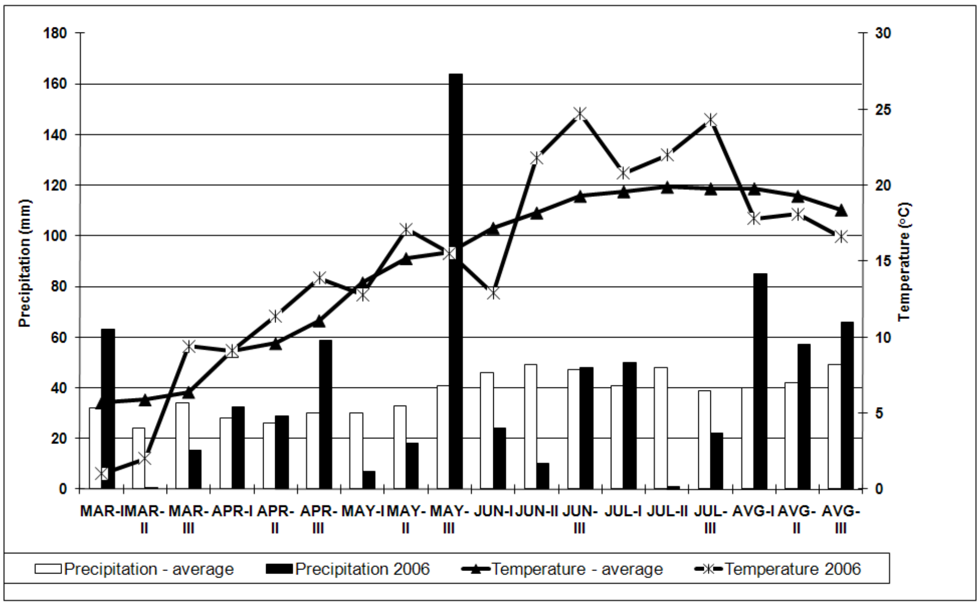

Figure 2: Precipitation amount and average decade's temperatures in growth season of hop in 2006 compared to long term average
Slika 2: Padavine in povprečne dekadne temperature v rastni sezoni hmelja v letu 2006 v primerjavi z dolgoletnim povprečjem

In 2006 season spring was cold and wet (Figure 2). There was a lot of precipitation at the beginning of June. After the warming, hop plants grew faster, but above-average temperatures in the second half of June and in July accompanied by a deficiency of precipitation slowed plant growth again. Plants went faster through the growth stages, photosynthesis was slowed down. After hot and dry July, a relatively cold and wet beginning of August followed.

3 RESULTS AND DISCUSSION

3.1 Proline content in hop leaves

3.1.1 Proline content in hop leaves – pot experiment

At the start of the experiment, in the middle of July 2005, proline content in hop leaves was from 0.90 mg/g dry matter (DM) in wild hop JUG2 to 1.77 mg/g in Southern Star. Hop varieties more sensitive to drought stress (Hallertauer Merkur and Cicero) had medium high proline content in leaves (1.45 mg/g and 1.01 mg/g DM respectively) (Table 1).

Table 1: Proline content in hop leaves (mg/g DM) in pot experiment in 2005
Preglednica 1: Vsebnost prolina v listih hmelja (mg/g suhe snovi) v lončnem poskusu v letu 2005

	18.7.2005	19.8.2005			20.10.2005		
	Initial value	WR*	WP	DS	WR	WP	DS
TOLERANT							
Aurora	1.06	0.24	0.29	8.32	0.22	0.90	0.27
Celeia	1.24	0.18	0.27	4.96	0.48	0.27	0.28
Taurus	1.43	0.09	0.29	4.97	0.26	0.39	0.19
Southern Star	1.77	0.25	0.27	2.77	0.03	0.70	0.11
JUG2	0.90	0.37	0.23	1.66	0.12	0.32	0.13
SENSITIVE							
Merkur	1.54	0.22	0.12	2.60	0.15	0.28	0.17
Cicero	1.01	0.30	0.26	3.30	0.25	0.46	0.21
NEW VARIETY AND HYBRID							
Dana	1.17	0.21	0.24	0.54	0.07	0.29	0.22
279/122	1.53	0.27	0.33	4.75	0.50	0.26	0.25

*WR = watered regularly, WP = watered with regard to precipitation outdoors, DS = drought stress simulation

In one month proline content in leaves decreased by more than 80 % if plants were watered and increased if plants were exposed to drought stress. In varieties more tolerant to drought it increased from 1.6 times in Southern Star to 7.8 times in Aurora. In drought more sensitive varieties Hallertauer Merkur and Cicero it increased by 1.7 and 3.2 times respectively. In hybrid 279/122 proline content increased 3.1-fold. The only exception was Dana variety (Table 1).

In the middle of August the highest proline content in hop leaves was detected in Aurora hop plants which were not watered since mid-July (8.32 mg/g DM). The value was also high in Celeia (4.96 mg/g) and Hallertauer Taurus (4.97 mg/g). Strong response to drought was also recorded in hybrid 279/122.

From mid-August (after we started watering again) to October proline content in the leaves drastically decreased in plants that were exposed to drought for one month in the summer (DS treatment) in all varieties. As a result of this the values in October were even lower compared to the leaves of plants that were watered in the summer. On the other hand, from mid-August to October proline content in the leaves of hop plants watered regularly in the summer (WR treatment) changed differently from variety to variety.

Next year, in July 2006, the highest proline content in the leaves of the majority of varieties was recorded in plants that were not watered for a month in the previous season – the content was from 0.47mg/g DM in Southern Star leaves to 2.88 mg/g DM in Hallertauer Taurus leaves. After two weeks of experiment the initial value of proline content in hop leaves decreased if plants were watered while in the leaves of non-watered plants proline content varied from variety to variety. There were no detectable differences between tolerant and sensitive varieties (no data provided).

3.1.2 Proline content in hop leaves – field experiment

At the beginning of experiment in July 2005 proline content in hop leaves of different varieties was relatively similar – it varied from 0.23 mg/g to 0.41 mg/g (Table 2). From mid-July to the end of August proline content in hop leaves increased mainly in Aurora (2.5-fold) and Hallertauer Taurus (1.6-fold).

Table 2: Proline content in hop leaves (mg/g DM) in field experiment in 2005 and 2006
Preglednica 2: Vsebnost prolina v listih hmelja (mg/g suhe snovi) v poljskem poskusu v 2005 in 2006

	19.7.05	2.8.05	23.8.05	18.10.05	18.7.06	2.8.06	18.8.06	9.10.06
TOLERANT								
Aurora	0.26	0.64	0.35	0.14	0.35	0.35	0.37	0.16
Celeia	0.37	0.55	0.38	0.24	0.58	0.25	0.24	0.37
Taurus	0.41	0.66	0.58	0.61	0.56	0.46	0.70	0.33
Southern Star	0.32	0.47	0.36	0.38	0.41	0.38	0.39	0.34
JUG2	0.32	0.51	0.40	0.51	0.60	0.57	0.43	0.27
SENSITIVE								
Merkur	0.35	0.44	0.43	0.27	1.25	0.42	0.42	0.40
Cicero	0.31	0.38	0.36	0.28	0.37	0.32	0.33	0.33
NEW VARIETY AND HYBRID								
Dana	0.23	0.38	0.36	0.15	0.34	0.24	0.53	0.20
279/122	0.36	0.53	0.29	0.20	0.56	0.18	0.47	0.14

From the beginning of August to mid August proline content decreased in the leaves of drought more tolerant varieties but remained unchanged in sensitive varieties. Proline content in hybrid 279/122 under the 2005 field conditions was comparable to drought more tolerant varieties, and in Dana more to less tolerant varieties (Table 2).

Mid-July 2006 was hot and dry. Proline content in hop leaves of all varieties was higher compared to the same period in 2005, which was rainy and colder. From mid-July to the beginning of August proline content in the leaves decreased or remained unchanged; no obvious differences among drought more or less tolerant varieties were detected. From the beginning of August to mid-August proline content in leaves remained unchanged in drought less tolerant varieties as in the previous season, but by contrast proline content in drought more tolerant varieties was not the same (Table 2). The response to dry conditions in the field was not as strong as in the pot experiment, or not detectable at all because the roots of hop plants can grow deep in the soil and absorb water from the lower soil layers therefore water deficits were not that high.

3.2 Potassium content in hop leaves

3.2.1 Potassium content in hop leaves – pot experiment

At the beginning of pot experiment, in mid-July 2005, the lowest potassium content in hop leaves was recorded in Hallertauer Merkur and Cicero, and in drought sensitive varieties (0.50 % and 0.61 % respectively). In drought more tolerant varieties potassium content in leaves was from 0.70 % in Hallertauer Taurus to 1.34 % in Aurora and Southern Star. In hybrid 279/122 it was in the same rank as in drought more tolerant varieties (0.84 %), in a new Slovenian Dana variety it was more similar to drought less tolerant varieties (0.56 %) (Table 3).

Table 3: Potassium content in hop leaves (% of DM) in pot experiment in 2005
Preglednica 3: Vsebnost kalija v listih hmelja (% v suhi snovi) v lončnem poskusu v letu 2005

	18.7.05	19.8.05		20.10.05	
	Initial value	WR*	DS	WR	DS
TOLERANT					
Aurora	1.34	0.57	1.18	0.84	1.55
Celeia	1.06	0.37	1.98	0.96	2.30
Taurus	0.70	0.67	1.48	0.61	2.06
Southern Star	1.34	0.70	1.06	0.77	1.15
JUG2	0.82	0.73	0.53	0.67	0.99
SENSITIVE					
Merkur	0.50	0.78	1.27	0.74	1.44
Cicero	0.61	0.75	1.22	0.86	2.49
NEW VARIETY AND HYBRID					
Dana	0.84	0.48	1.20	0.49	1.45
279/122	0.56	0.65	1.30	1.35	1.42

*WR = watered regularly, DS = drought stress simulation

If plants were watered, potassium content decreased in leaves of drought more tolerant varieties after a month, but increased a bit in drought less tolerant ones (Table 3). The response of Dana cultivar was comparable to tolerant varieties, and the response of 279/122 to drought less tolerant. When plants were left without water, potassium content increased more in leaves of drought less tolerant varieties compared to the plants of the same varieties that were watered. The response of drought more tolerant varieties to no watering conditions was different; in some drought more tolerant varieties (Aurora, Southern Star, JUG2) proline content in leaves decreased, but increased in Celeia and Hallertauer Taurus.

In mid-August the value was higher in the leaves of non-watered plants compared to the watered ones (with exception of JUG2) (Table 3). In drought sensitive varieties Hallertauer Merkur and Cicero potassium content was 1.6-fold higher in the leaves of non-watered plants compared to the leaves of watered plants, and in drought more tolerant varieties from 2.0-fold to 2.6-fold higher. This trend was not recorded in wild JUG2 and Southern Star which showed similar response as drought less tolerant varieties (Table 3).

In the time of regular watering which started in mid-August potassium content in the leaves of plants that were not watered for a whole month increased or remained unchanged until October. Potassium content in leaves of watered plants remained almost unchanged in the majority of varieties and increased in some (Aurora, Celeia and 279/122) (Table 3).

In mid-July of 2006, potassium content in the leaves of plants that were not watered for a month in the previous season was similar or higher compared to the watered ones. The only exception was Southern Star where the value was similar. At that time the experiment started again; we stopped or continued watering the same plants as in the previous season. After two weeks potassium content in the leaves of plants that were not watered increased from 1.1-fold in Hallertauer Taurus to 2.4-fold in Celeia. The only exception was Aurora where potassium content remained unchanged. Among drought less or more tolerant varieties no differences in response of plants regarding potassium content in the leaves were recorded. In the leaves of watered plants potassium content remained unchanged or decreased by more than a half in Southern Star and JUG2. From the beginning to mid-August potassium content in leaves decreased by half in all varieties (data not provided).

3.2.2 Potassium content in hop leaves – field experiment

There was no water deficiency in the field in the summer of 2005. The season was wet with not very high temperatures. In mid-July potassium content in the leaves of drought sensitive varieties was higher (from 1.05 % in Hallertauer Merkur to 1.23 % in Cicero) compared to drought more tolerant varieties (from 0.51 % in Southern Star to 0.93 % in JUG2) (Table 4), which was just the opposite to the pot experiment results (Table 3). The values increased by the beginning of August and exceeded the values of drought sensitive varieties.

Table 4: Potassium content in hop leaves (% in DM) in field experiment in 2005 and 2006
Preglednica 4: Vsebnost kalija v listih hmelja (mg/g suhe snovi) v poljskem poskusu v 2005 in 2006

	19.7.05	2.8.05	23.8.05	18.10.05	18.7.06	2.8.06	18.8.06	9.10.06
TOLERANT								
Aurora	0.60	1.62	0.89	0.33	1.07	1.06	0.69	0.28
Celeia	0.89	1.07	0.72	0.20	1.35	1.08	0.94	0.45
Taurus	0.52	1.78	0.88	0.77	0.69	0.50	0.93	0.36
Southern Star	0.51	1.59	0.87	0.91	0.70	0.46	0.53	0.41
JUG2	0.93	1.01	0.85	0.77	0.63	0.49	0.43	0.38
SENSITIVE								
Merkur	1.05	0.99	1.00	0.72	0.46	0.35	0.56	0.34
Cicero	1.23	0.96	0.96	0.26	1.03	1.07	0.41	0.42
NEW VARIETY AND HYBRID								
Dana	1.22	1.62	0.75	0.47	0.40	0.73	0.52	0.44
279/122	1.41	1.90	0.80	0.96	0.71	0.41	0.53	0.38

From mid-July to the beginning of August potassium content increased most in the leaves of Hallertauer Taurus (to 1.78 %), Southern Star (to 1.59 %) and Aurora (to 1.62 %). From the beginning to mid-August potassium content decreased in drought tolerant varieties and remained unchanged in drought sensitive ones. Genotypes 279/122 and Dana variety showed increased potassium content in leaves as it was detected in drought more tolerant varieties while initial values (in mid-July) were more comparable to drought sensitive varieties. From mid-August to October the values remained unchanged, or they decreased (Table 4).

Year 2006 was dry with very high temperatures in the second half of July and relatively cold and rainy in the first half of August. Potassium content in the leaves of drought more tolerant varieties was higher compared to the same period of the previous year but lower in drought sensitive varieties. In two weeks when dry weather finally prevailed (mid-July to the beginning of August) no obvious differences were recorded between more or less drought tolerant varieties.

From the beginning of August by October potassium content decreased in the majority of varieties (Table 4).

4 CONCLUSIONS

Higher potassium content in leaves was recorded in hop plants under drought stress conditions compared to adequate water supply, but the values differed from variety to variety. In July, potassium content in the leaves of drought more tolerant varieties was higher in drier season compared to the season with enough precipitation but lower in drought sensitive varieties.

From July to August proline content in leaves decreased by more than 80 % if plants were watered regularly and increased in the leaves of plants that were exposed to water stress. In varieties that are considered to be tolerant to drought stress it increased from 1.6-fold in Southern Star to 7.8-fold in Aurora. In sensitive varieties proline content increased from 1.7-fold in Hallertauer Merkur to 3.2-fold in Cicero (to 2.6 mg/g and to 3.3 mg/g respectively).

Preliminary results can be a basis for further investigations since no clear boundary was detected among the varieties.

The investigation showed biochemical markers that could contribute to faster selection of drought stress tolerant hybrids and the development of selection method, but investigations will continue in the future.

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