

DEVELOPMENT OF SOYBEAN VARIETIES WITH SPECIFIC NUTRITIONAL COMPOSITION OF GRAIN

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Abstract

The major limitation to use row soybean grain in animal feed is presence of protease inhibitors - Kunitz trypsin inhibitor (KTI) and Bowman - Birk inhibitor (BBI), the main anti-nutritional factors of soybeans. They are responsible for the reduced digestibility of seed proteins and may cause disruption in animal's development. About 80% of tryptic activity inhibition is caused by KTI. Grain of conventional soybean varieties requires heat processing to break down trypsin inhibitor's activity before using as food or animal feed. The excessive heat treatments (uncontrolled temperature; long period of time) may decrease protein solubility and lower amino acid availability. The genetic control of presence of (Ti) has been reported as a co-dominant multiple allelic series at a single locus, while lack of KTI is inherited as a recessive allele designated ti.

A section of soybean breeding program in Maize Research Institute Zemun Polje is aimed at reducing trypsin inhibitor activity, by crossing parent donor of desirable character (variety Kunitz-titi line) with adapted high yielding varieties (Ti line). Presence/absence of KTI in progeny was done by protein electrophoresis of mature seed from the individual plants in several segregating generations. After field trials over years and different locations, high-yielding soybean lines lacking KTI were identified.

As a result of this breeding program, two varieties lacking KTI - Lana (maturity group II) and Laura (maturity group I), were released. Trypsin inhibitor content ranged from and 15.01 mg g⁻¹ in Laura to 15.35 mg g⁻¹ in Lana, which was about 50% less than in the genotypes of standard grain type. Grain yield of varieties is equal to high yielding varieties from the same maturity groups. Soybean lines with reduced protease inhibitor content could reduce or eliminate the need for expensive heat treatments and lessen the chance of lowering amino acid availability.

This type of varieties might be suitable for small farms with direct feed production and animal growing.

Key words: Soybean, Breeding, Kunitz trypsin inhibitor, Animal feed.

1. Introduction

Soybean is considered as a one of the best sources of valuable high quality protein for human consumption and animal feed. The protease inhibitors in soybean, Kunitz trypsin inhibitor (KTI) and the Bowman-Birk inhibitor (BBI) constitute at least 6% of the protein present in soybean seed (Ryan [1]) and with lectin, represent the main anti nutritional factors of soybeans. Major of the trypsin inhibition is caused by KTI (Brandon [2]), which strongly inhibits trypsin, and therefore reduces protein digestibility and food intake. Ingestion of raw soybean meal causes hypersecretion of pancreatic enzymes, leading to demands of sulphur-containing amino acids and resulting in growth depression of monogastric animals.

Due to this, row soybean cannot be used for animal feeding and needs to be heat-processed to eliminate thermolabile anti nutritional factors. Numerous studies investigated the effect of soybean variety and processing on growth performance of pigs (Cook *et al.* [3], Palacios *et al.* [4]). Heat processing inactivates anti-nutritional factors and modifies the structure of the proteins, making them more available for digestion. Despite the efficiency of thermal treatment to reduce protease inhibitors, residual inhibition (10 - 20%) is maintained (Carvalho *et al.* [5]). Furthermore, excessive heat treatments (uncontrolled temperature; long period of time) may cause quality changes in soybean grain, resulting in decreased protein solubility and low amino acid availability. Soybean lines with reduced

protease inhibitor content could reduce or eliminate the need for expensive heat treatments and lessen the chance of lowering amino acid availability.

The genetic control of presence of KTI (allele *Ti*) has been reported as a co-dominant multiple allelic series at a single locus, while lack of KTI is inherited as a recessive allele designated *ti*. A section of soybean breeding program in Maize Research Institute Zemun Polje is aimed at developing cultivars with reduced trypsin inhibitors content in mature grain, by crossing parent donor of desirable character (variety Kunitz-*titi* line) with adapted high yielding varieties (*Ti* line).

As a result of breeding for this trait, two Kunitz trypsin inhibitor-free (KTI free) varieties Lana and Laura were released. Total trypsin inhibitor (TI) content in new cultivars was about 50% reduced as compared to the conventional cultivars (standard grain type). Variety Lana was released in year 2004 and belongs to maturity group II (late cultivar). Lana is characterized by good agronomic performances (high genetic potential for seed yield, resistance to lodging and seed shattering, satisfactory field resistance to major diseases and adapted to the growing conditions in our region) and total TI content 15.01 mg g⁻¹. Three years later, as a result of the same breeding program, another KTI-free soybean cultivar named Laura was released. This study presents breeding method for developing cultivar Laura and its productive and nutritional characteristics. KTI-free soybean cultivars may solve the problem of direct livestock nutrition and make food processing more economical.

2. Materials and Methods

In order to eliminate KTI as and anti-nutritional factor in soybean grain, a cross between varieties Kunitz and Novka has been made. Kunitz is late cultivar (maturity group III), first registered in USA as a variety lacking KTI (Bernard *et al.* [6]). Novka is domestic variety from maturity group I, obtained in exchange of breeding material among the institutions in the region. Presence/absence of KTI in progeny was done by polyacrylamide gel electrophoresis of mature seed from the individual plants in several segregating generations according to Hymowitz and Hudley procedure [7]. Lines identified as KTI-free were tested in field trials. After yield testing, one perspective line from the cross combination Kunitz x Novka lacking KTI was selected and denoted L 94 128.

The line is submitted for the registration in year 2005. According to the criteria for the registration of newly created soybean varieties, the line with specific trait is being tested under the same conditions as the material of standard grain type. The requirement for new variety to be released is it to be equal in seed yield with the cultivars set up as standard in the corresponding maturity group.

The line L 94 128 was tested during the year 2005 and 2006 in the network of field experiments conducted by a Department for plant variety registration and protection, Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Field trials were set on five locations, with a cultivar Balkan as a standard. To introduce the new variety, the results of 4-years investigation in small plot trials at the experimental fields of Maize Research Institute "Zemun Polje" are presented. Examined cultivars are grown in a randomized complete block experiment with two replicates and two rows per genotype, during the years 2002 - 2005. Following traits were scored: seed yield (kg/ha), number of days to maturity, lodging (1 - 5 scale) and 1000 grain weight (g). Comparison of mean seed yield of L 94 128 and standard cultivar Balkan was performed using LSD (least significant difference) test. Laboratory testing program included: determination of total TI content according to modified Erlanger method, as described by Hamerstrand *et al.* [8], using Na-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) as substrate; analysis of protein content according to Kjeldahl method and total oil content determined by the diethyl ether-based Soxhlet extraction procedure.

The line L 94 128 was released in year 2007, under name Laura.

3. Results and Discussion

Protein electrophoresis is the most common method to select lines lacking KTI. Identification of KTI-free soybean lines from the cross Kunitz x Novka was done by polyacrylamide gel electrophoresis of mature seed from the individual plants in several segregating generations. Figure 1 shows parental cultivars (Kunitz, Novka) and seven lines from F₄ generation, segregating for the presence or absence of KTI. Lines that possess KTI protein (*Ti* lines) had 21.5 kDa band that indicates presence of KTI protein, while KTI-free lines (*titi* lines) did not have the band in protein gel electrophoresis from the mature seed. Lines L 94 128 and L 7 were identified as KTI-free lines and subjected to yield testing in field trials and evaluation of total trypsin inhibitor content in laboratory analysis. Line L 7 was excluded from the trials after first year of testing, due to bad agronomic performances.

During the period 2002 - 2005, the line L 94 128 has been tested in various types of field experiments at multiple locations. In order to represent the variety, results obtained in small-plot trials at experimental fields of Maize Research Institute "Zemun Polje" are shown in Table 1.

Seed yield is the main indicator of the productivity of cultivar. Seed yield data were processed for each year separately, due to differences in field trials set up.

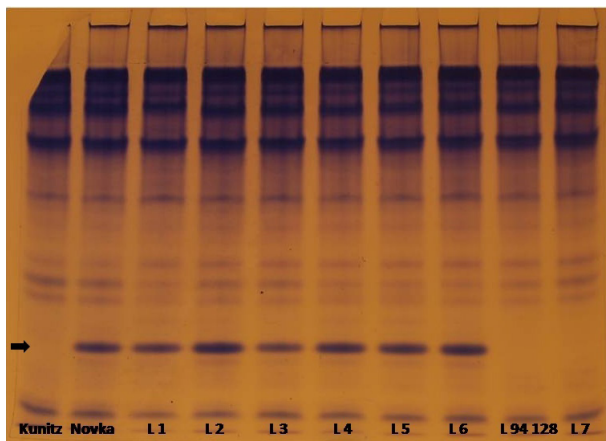


Figure 1. Polyacrylamide gel of protein extracted from seeds of parents and F4 progeny; arrow points to the Kunitz trypsin inhibitor band (21.5 KDa)

Table 1: Agronomic traits of new cultivar Laura and standard Balkan in small-plot trials at the experimental fields of Maize Research Institute Zemun Polje during 2002-2005

Variety	Year			
	2002	2003	2004	2005
Seed yield (kg/ha)				
Balkan	4356	3637	4277	3780
Laura	4441	3624	4139	3868
Lsd 0.05	212	198	228	227
Lsd 0.01	243	236	287	299
Number of days to maturity				
Balkan	136	140	135	139
Laura	138	143	138	142
Lodging (1-5)				
Balkan	2	1.5	2	1.5
Laura	1.5	1	1.5	1
1000 grain weight (g)				
Balkan	205.3	208.0	206.4	206.7
Laura	196.1	196.9	196.4	196.8

According to the results of LSD test, differences in seed yield between variety Laura and standard variety Balkan were not significant in all years of study. In years 2002 and 2005, cultivar Laura achieved 85 and 88 kg/ha higher seed yield compared to cultivar Balkan, respectively, while during years 2003 and 2004 Laura had yielded less than standard. However, requirement for registration of the variety has been met, given that the varieties with specific properties need to be at the same level with the standard, in order to be released.

A very important trait of the soybean variety is vegetation length, since it determines a particular region in which one variety could be grown.

Variety Laura belongs to mid season soybean varieties, with average number of 140 days to maturity. Growing season length of Laura is appropriate for the maturity group I. Variation in number of days to maturity compared to the standard of the maturity group I was maximum 3 days, fulfilling the condition for belonging to the same maturity group.

Variety Laura is characterized by complete absence or very weak lodging, while seed shattering was not noted in any of research years.

A thousand grain weight is lower as compared to the standard. This property has a direct impact on the yield, because it represents one of the three main yield components (Đukić *et al.* [9]). It is genetically determined, but also strongly influenced by agro-ecological conditions during the seed filling period.

Table 2: Seed quality parameters of new cultivar Laura and standard Balkan determined in laboratory for technology of Maize Research Institute Zemun Polje during 2002-2005

Variety	Year			
	2002	2003	2004	2005
Protein content (%)				
Balkan	37.15	36.42	37.21	36.36
Laura	37.92	37.12	38.03	37.11
Oil content (%)				
Balkan	20.23	20.40	20.26	20.38
Laura	19.62	19.95	19.55	19.84
Total trypsin inhibitor (TI) content (mg/g)				
Balkan	30.30	30.65	31.40	31.05
Laura	14.15	15.25	16.10	15.90

Soybean contains 38 to 40% good quality protein and approximately 19% oil, expressed on dry matter basis (Wilson [10]), which make this crop the most important protein and oilseed source worldwide. Laura has a higher protein content and lower oil content as compared to a standard (Table 2). This is in accordance with numerous findings about negative correlation between oil and protein content in soybean (Burton [11]; Cober and Voldeng [12]).

Control of the presence of KTI was carried out through the analysis of the total trypsin inhibitor content. The investigated soybean varieties displayed different total TI level. As expected, the lowest TI content was detected in Laura, KTI-free cultivar. Genotype Balkan containing the KTI protein showed a higher TI level. In this way the samples can be divided into two distinct classes, where it is clear that the consequence of the lack of KTI is a lower content of the total TI. Residual inhibition present in both KTI-free (Laura) and standard cultivar

(Balkan) is due to the Bowman-Birk trypsin inhibitor, responsible for the rest of tryptic activity inhibition. Previous results suggest that simple removal of Kunitz inhibitor without any intervention upon Bowman-Birk inhibitor, although allowing a reduction of processing costs (Friedman *et al.* [13]) does not appear to solve a problem of direct livestock nutrition.

4. Conclusions

- Soybean variety Laura is released as a one with specific properties – lacking Kunitz trypsin inhibitor in mature grain. Thanks to that, trypsin inhibitory activity was reduced to approximately half as compared to the standard grain quality varieties.

- Row grain of cultivar Laura can be used as animal feed for adult non-ruminants, without previous heat treatment. In industrial processing, procedure can be done in shorter time, with saving energy. Laura belongs to the first maturity group and is suitable for all growing regions. This cultivar does not require any specific conditions of cultivation compared to the standard grain quality varieties.

- Although the nutritional value of raw Kunitz trypsin inhibitor-free soybean varieties is diminished by other heat-labile factors present in grain, such varieties might be processed more economically with shorter heating time and lower temperature, or utilized in extensive farming systems.

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