



Ecocycles, Vol. 5, No. 2, pp. 24-32 (2019)
DOI: 10.19040/ecocycles.v5i2.148

ORIGINAL ARTICLE

Horticultural uses of botanical variations of woodland sage (*Salvia nemorosa* L.)

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Abstract – Woodland sage (*Salvia nemorosa* L.) is an „underestimated” plant at present, but it may be a promising plant species for the future. This is especially true in the Central and Eastern Europe, a region exposed to certain climate changes, such as the more extreme weather, the fluctuation of rainfall distribution, the increasing of temperature, the rapid and unfavorable changes of climate in densely populated cities, the heat-island effects, the atmospheric drought, etc. Therefore, *Salvia nemorosa* seems to be a very promising plant for future generations. The searching and collecting program of the wild botanical variants of woodland sage were launched in 2009, as part of two botanical expeditions carried out in Hungary.

Keywords – climate change, woodland sage, *Salvia nemorosa* L., wild botanical variations, horticultural use

Received: October 22, 2019

Accepted: December 7, 2019

1. INTRODUCTION

Woodland sage, as an ornamental plant has a considerable drought-tolerant capability (Kaprinyák et al. 2012). A botanical expedition launched in 2009 to search and collect the *lusus* forms of woodlands sage discovered by the famous Hungarian ornamental breeder, Zoltán Kováts in the years of 1930s. The next expeditions were organized in 2010 and in 2011. The main aim of these expeditions was searching for natural woodland sage populations located in Hungary and their botanical description of the valuable versions, as well as study of their hereditary characteristics, flowering possibilities (Váradi 2013).

2. MATERIALS AND METHODS

2.1. Location of the experiment, geographical condition

2.1.1. The original habitat of *Salvia nemorosa* variation

Figure 1 shows the Sites of *Salvia nemorosa* variation in Gáborján (Kováts 2010).

2.1.2. Location of the field experiment, condition

The field experiments were carried out at the Plants for the Future Biomass Demonstration Garden of the University of Debrecen, where the soil properties were previously analyzed (Table 1). The mother plants were originated from the flood

zone of Berettyó river. Applying these mother plants and clones of the varieties, developing by splitting, were further propagated under field conditions. Because of the additional water supplementing was realized here by flood-irrigation, we transferred the strong-rooted woodland sage variations in blocks cassette. Five plants were places into one cassette, all-together 25 plants of different colors were planted in our experiment plot. The plants were spaced 50 x 50 cm distance from each other (Figure 2.).

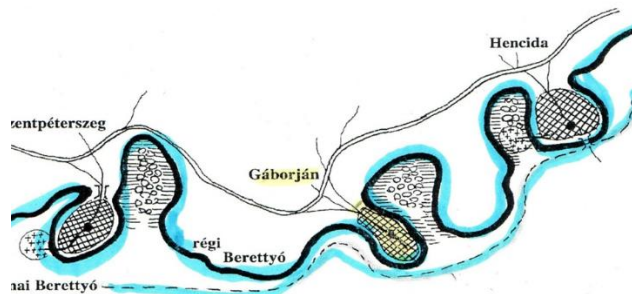


Figure 1. Sites of *Salvia nemorosa* variation (Gáborján, 2009, Kováts 2010)

Table 1. Chemical and biochemical properties of the soil at the Plants for the Future Biomass Demonstration Garden of the University of Debrecen (Alshaall, 2013)

| Soil properties or parameters | Value |
|---|-------|
| pH | 7.63 |
| Electrical conductivity (dS m ⁻¹) | 0.45 |
| Soil organic carbon (g kg ⁻¹) | 19.14 |
| Dehydrogenase (µg TPF g ⁻¹) | 30.20 |
| Phosphatase (mgP ₂ O ₅ /100g soil/2h) | 0.54 |
| Urease (NH ₄ ⁺ mg/100g soil) | 363 |
| Catalase (O ₂ ml/2min) | 13.0 |
| Total bacterial count (x 10 ⁷ CFU/g) | 1.24 |
| Total fungi count (x 10 ⁵ CFU/g) | 0.56 |

2.2. Method of experiments

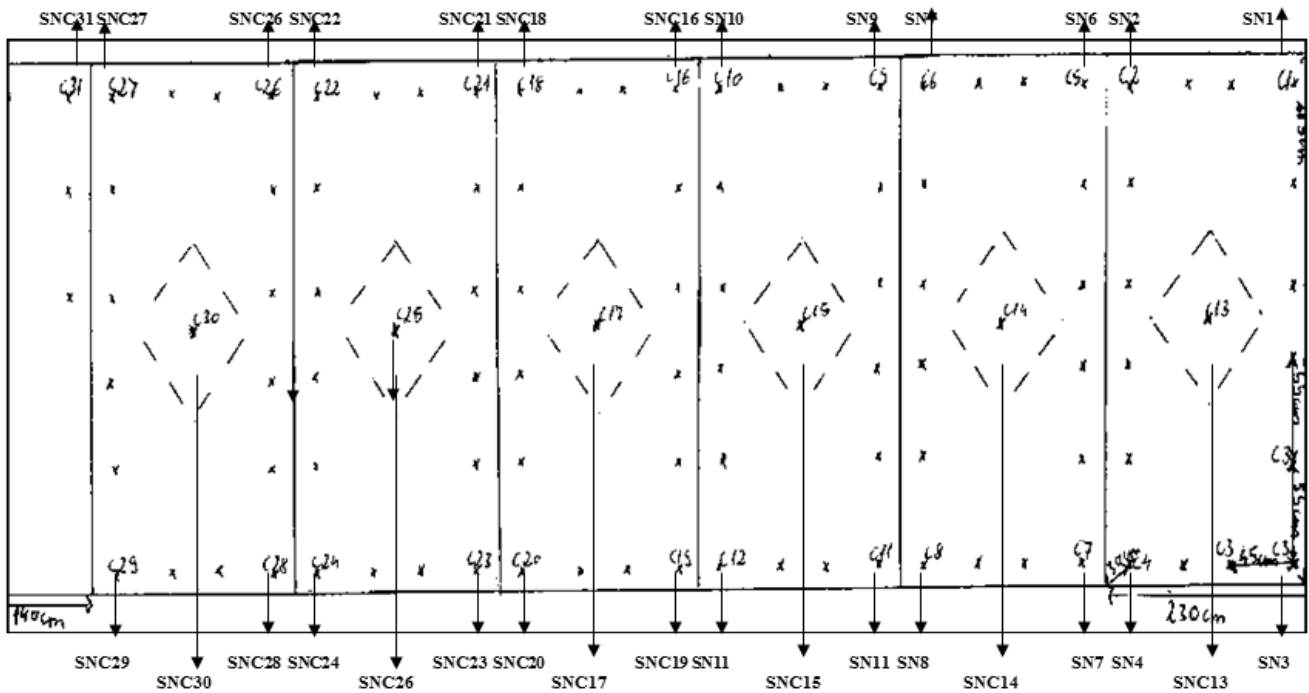


Figure 2. Field planting map of *Salvia nemorosa* L. botanical variations, (University of Debrecen MÉK, Plants for the Future Biomass Demonstration Garden 2011)

2.2.1. Establishment of the field population

In 2010, by the guidance of Zoltán Kováts, the members of our team organized an expedition for mapping some natural woodland sage population located in Hungary. The exploratory work continued in the next year in June, at the end of the flowering phenological phase. After cultivating the broke cuttings in plots, the mother plants and clones were grown under field conditions. In 2013, 12 varieties were picked up from the variations having high shape and color

aesthetic value by positive selection and they were individually placed in order to prevent seed collecting.

2.2.2. Botanical measurements of the selected variations

In 2013, we measured the plant height, habit, length of inflorescence axis, number of inflorescence per axis, leaf color, and the upper and lower lip's color (Váradi 2013). The individual stamps were continuously recorded in the period of flowering and counted the amount of the flowers per plant.

2.3. Molecular genetics methods

2.3.1. Comparison of ploidy level in woodland sage variations by flow-cytometry

After collecting the shoot apices originated from the field population, the sample was used on a 1 cm² leaf surface. Measurement device: Becton Dickinson FAC Scanflow cytometer (Lisztes-Szabó 2015). The calculations were conducted according to the database of the Kew Royal Botanical Gardens (I1, Table 2).

Table 2. Ploidy level of *Salvia nemorosa* according to the Kew Royal Botanic database (I1)

| Plant group | Species | Ploidy level | 2C (pg) |
|-------------|------------------------|--------------------|---------|
| Angiosperm | <i>Salvia nemorosa</i> | | 1.09 |
| C Mean | C Min. C Max. | Standard deviation | |
| 2C (pg) | 1.09 1.09 | 0.00 | |

2.4. Evaluation and analysis of experimental data

Applied statistical method: ROPstat 2.0, date of the last significant revision: June of 2011 (Vargha, 2011).

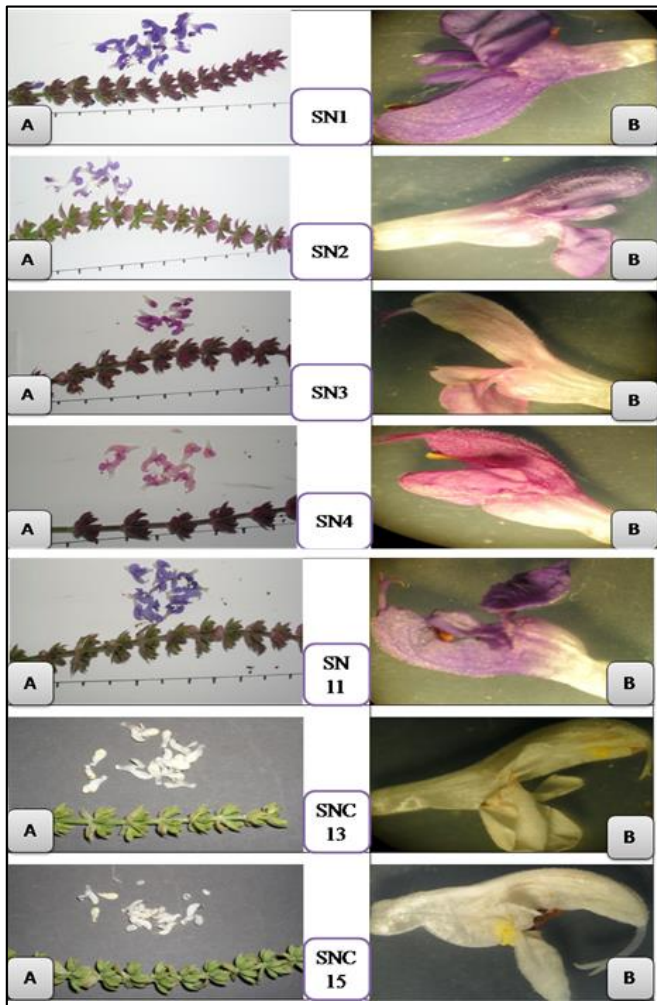


Figure 3/a. Inflorescence axis (A) and petal appearance (B) of *Salvia nemorosa* variations (— = 1 cm)

3.1. Botanical evaluation of valuable *Salvia nemorosa* variations (2012-2014)

3.1.1. Evaluation of woodland sage variations in aspect of horticultural usability

According to the literature, woodland sage has extremely broad morphological botanical variability (Natarajan and Kuchny, 2008). Tables 3/a, 3b, and Figures 3/a and 3b. illustrate the broad genetic variability of our newly collected variations.

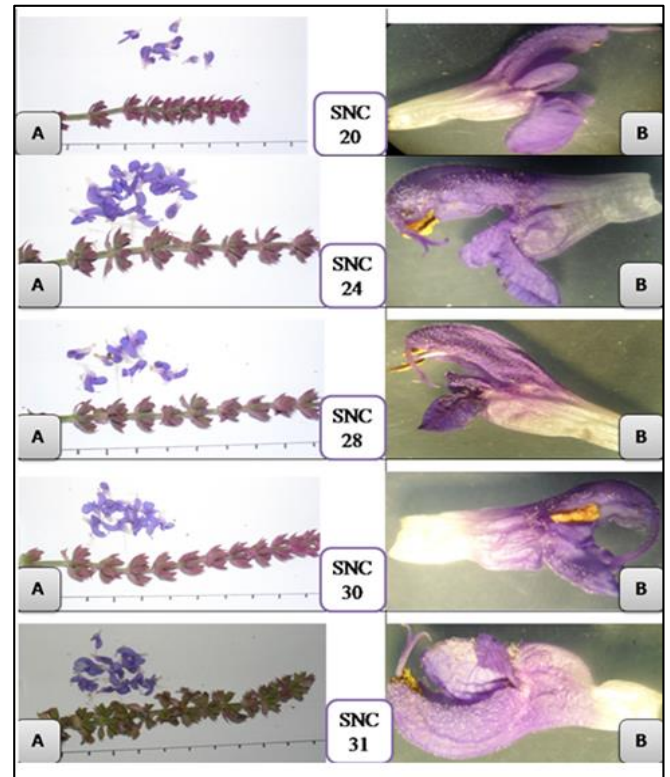


Figure 3/b. Inflorescence axis (A) and petal appearance (B) of *Salvia nemorosa* variations (— = 1 cm)

Table 3/a. Evaluation of different woodland sage varieties (M=medium, L=long, S=short, G=green, LG=light green, DG=dark green, B = blue, LB=light blue, PP=purple, LPP=light purple, PS=purple stripe, PSP=purple spots, W=white, P=pink, LP=light pink, GR=greyish, H=high, BL=big lip)

| | SN1 | SN2 | SN3 | SN4 | SN5 | SN6 | SN7 | SN8 | SN9 | SN 10 | SN 11 | SN 12 | SNC 13 | SNC 14 | SNC 15 | SNC 16 |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|-------|--------|--------|--------|--------|
| Inflorescence axis length | M | M | M | L | M | S | | | | | | | M | S | S | M |
| Inflorescence axis colour | G | PSP | P | G | G | G | | | | | | | G | G | G | G |
| Branched | x | | | H | | | | | | | | | | x | | |
| Inflorescence compact | | | | | x | x | | | | | | | | x | x | |
| Leaf colour | G | G | G | LG | G | G | | | | | | | LG | LG | LG | G |
| Upper lip colour | B | LPP | PP | LP | LB | PP | | | | | | | W | W | W | LPP |
| Lack of upper lip | x | | BL | | | | | | | | | | | | x | |
| White spots on upper lip | | | | | | x | | | | | | | | | | |
| Lower lip colour | B | LPP | P | LP | LB | PP | | | | | | | W | W | W | LPP |
| Lack of lower lip | x | | BL | | | | | | | | | | x | | | |
| White spots on lower lip | | | | | x | x | | | | | | | | | | |
| Sepal colour from above | PP | PP | PP | P | PP | PP | | | | | | | DG | GR | LG | G |
| Sepal colour from below | PP | PP | PP | P | PP | PP | | | | | | | G | LG | LG | G |
| Bract colour from above | PP | PPS | P | LPP | LG | PPS | | | | | | | LG | GR | GR | PP |
| Bract colour from below | PP | PPS | PP | P | G | PPS | | | | | | | G | G | G | PP |

Table 3/b. Evaluation of different woodland sage varieties (M=medium, L=long, S=short, VS=very short, G=green, LG=light green, DG=dark green, B=blue, LB=light blue, DB=dark blue, PP=purple, LPP=light purple, PS=purple stripe, PSP=purple spots, PPE=purple edge, W=white, P=pink, LP=light pink, GR=greyish, H=high, SM=small, BL=big lip, TL=tousled)

| | SNC 17 | SNC 18 | SNC 19 | SNC 20 | SNC 21 | SNC 22 | SNC 23 | SNC 24 | SNC 25 | SNC 26 | SNC 27 | SNC 28 | SNC 29 | SNC 30 | SNC 31 |
|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Inflorescence axis length | S | M | L | S | M | S | M | S | S | S | M | M | VS | S | S |
| Inflorescence axis colour | G | GR | G | G | G | G | G | G | G | G | PPS | G | PPS | G | G |
| Branched | | | | | | | | x | | | | | | | |
| Inflorescence compact | x | | | TL | | x | | x | | x | | | x | x | x |
| Leaf colour | G | G | G | G | G | G | G | G | G | G | G | G | G | G | G |
| Upper lip colour | W | LP | P | P | LPP | B | DB | LB | B | LPP | DB | LB | LPP | LB | LB |
| Lack of upper lip | | | | | SM | x | | | | | | | | | |
| White spots on upper lip | | | | | | | x | | x | | x | x | | | |
| Lower lip colour | W | LP | P | P | LPP | DB | DB | LB | B | LPP | DB | LB | PP | LB | LB |
| Lack of lower lip | | | | | | | | | | | | | | | |
| White spots on lower lip | x | | x | | | | | | | x | | | | | |
| Sepal colour from above | G | LPP | PP | LPP | LPP | PP | LPP | PP | LPP | LPP | LPP | PP | PP | PP | PP |
| Sepal colour from below | G | PP | PP | PP | PP | PP | LPP | PP | PP | PP | PP | PP | PP | PP | PP |
| Bract colour from above | GR | LPP | PP | PP | PP | PP | LPP | PP | PP | PPS | PP | PP | PPS | PPE | PP |
| Bract colour from below | G | PP | PP | PP | PP | PP | PP | PP | GR | PP | PP | PP | PPS | PPE | PP |

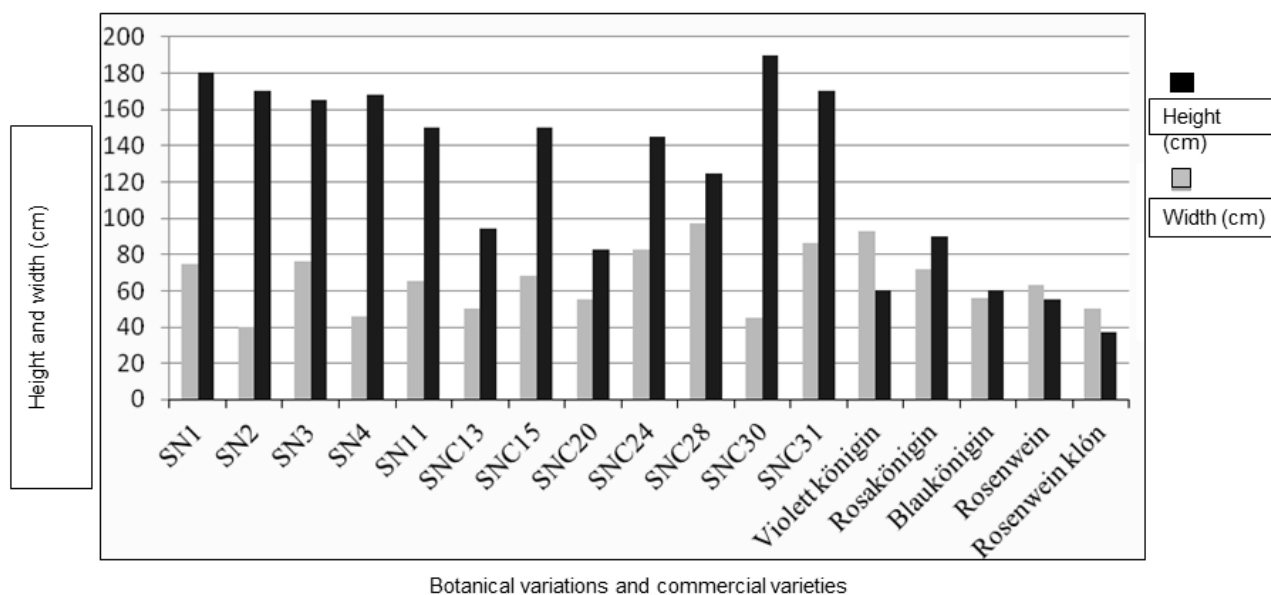


Figure 4. Height and width parameters of *Salvia nemorosa* lines / varieties (2014)

3.1.2. Comparison parameters of woodland sage botanical variations

Figure 4 shows variations according to the comparison of their height and width. Twelve valuable woodland sage botanical variations were found according to habit in 2014 (Figure 5):

- Erect habit: SNC3, SNC13, SNC20
- Half-postrate habit: SN1, SN11, SNC15, SNC24, SNC28, SNC31
- Postrate habit: SN2, SN4, SNC30

In 2014, the shoot developing tendency was measured, which represents the number of the stems located on one plant.

Variation SNC30 was significant (Figure 6); meanwhile, the stem developing value of clone SNC20 and clone 'Rosenwein' was equal. Figure 7. shows the differences between the main inflorescence axis length of the varieties and cultivars. The maximum value was measured in case of the 'Violett Königin' and SNC24 clone. Among the botanical variations the smallest main inflorescence axis length was recorded in case of SN4 and SNC31 clones, among the cultivars the smallest values were measured in the case of 'Blauköningin' and 'Rosenwein' cultivar. Based on the comparison of main inflorescence weight (Figure 8) the SN2 and SN3 variations showed the highest weight and the main inflorescence of the SNC20 was the smallest. The inflorescence weight of 'Rosenwein' clone variations is appreciable in comparison with the 'Rosenwein' cultivar.

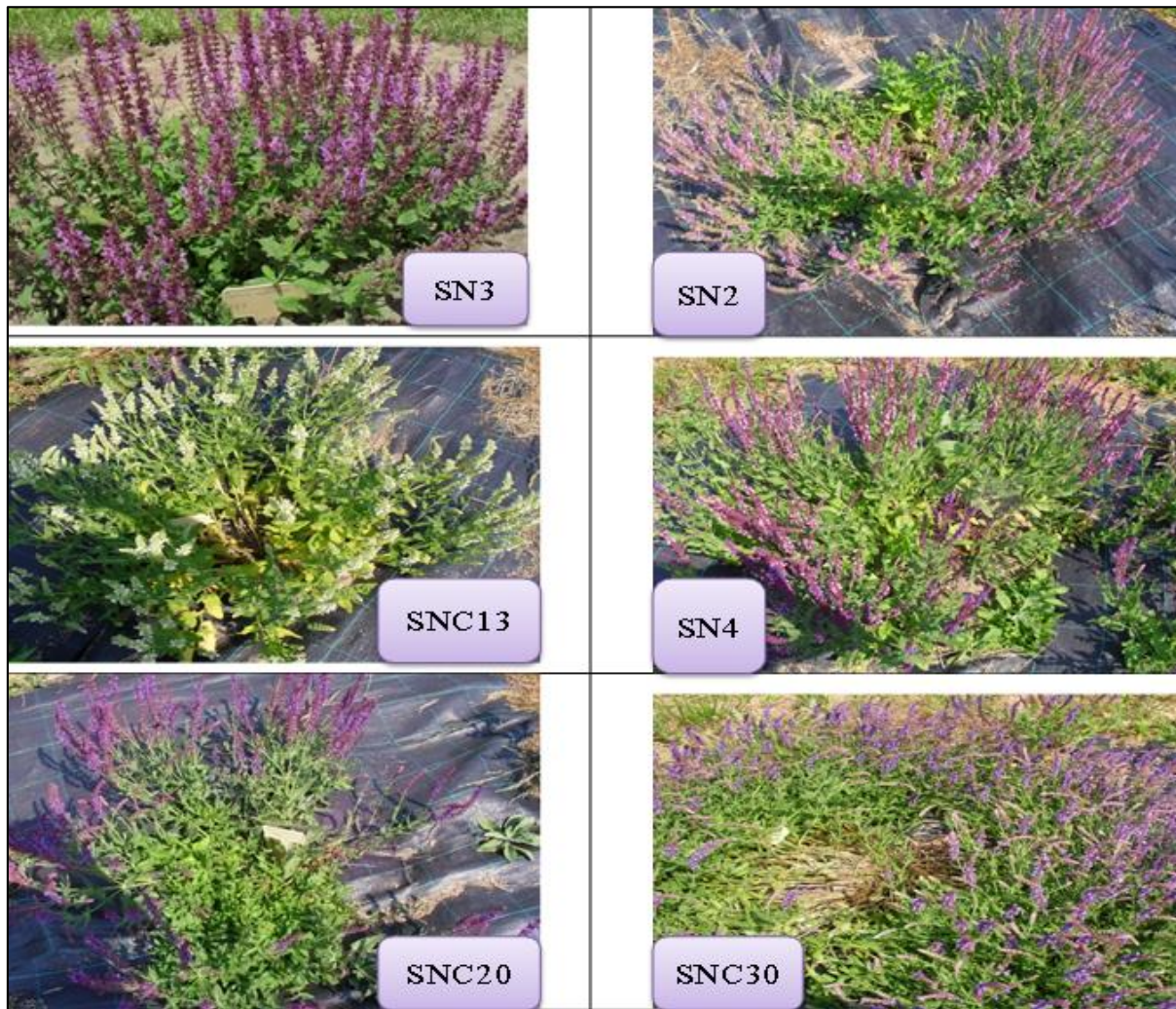


Figure 5. Habit of valuable *Salvia nemorosa* botanical variations (2014)

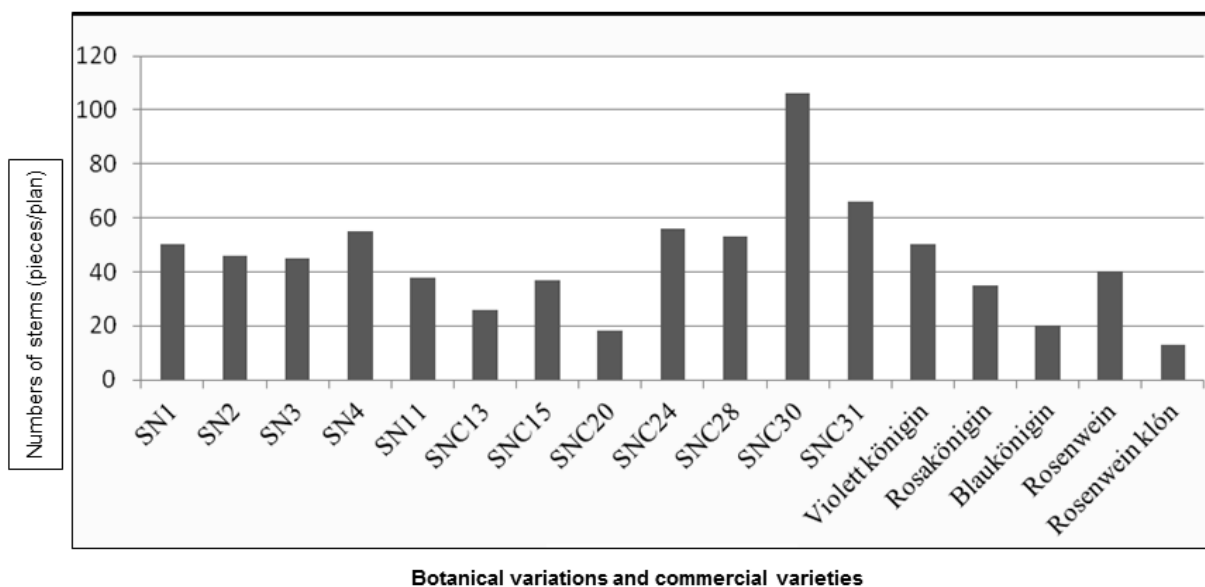


Figure 6. Stem development tendency of woodland sage botanical variations and cultivars in 2014 (pieces/plant)

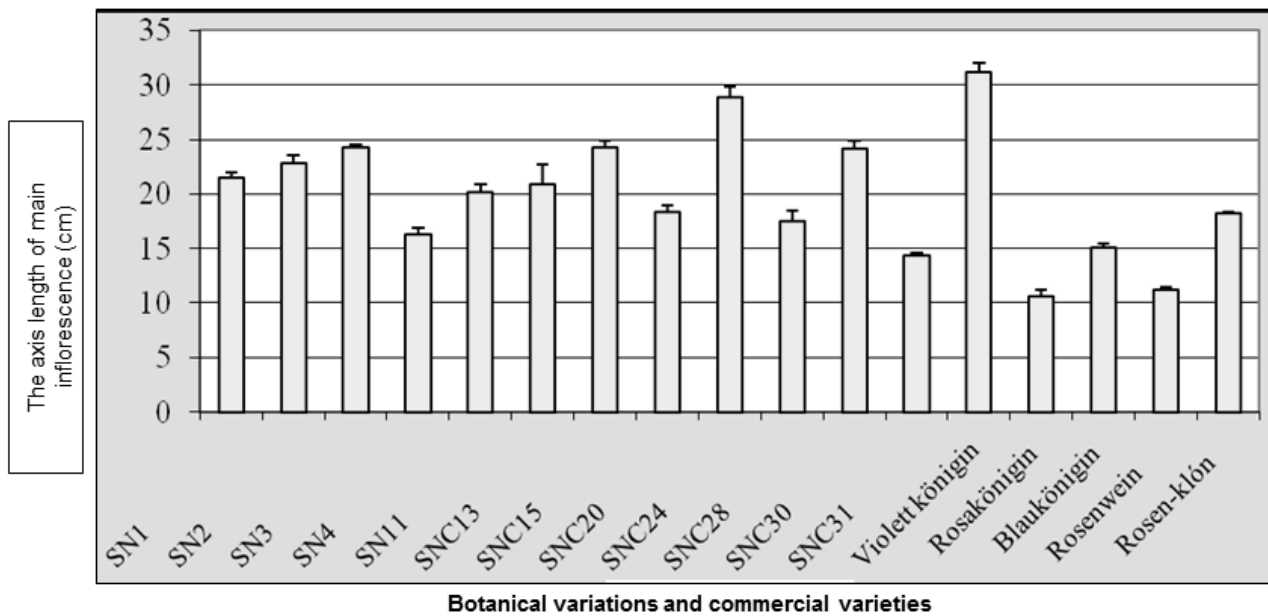


Figure 7. The axis length of main inflorescence in *Salvia nemorosa* cultivars and vain 2014 (cm)

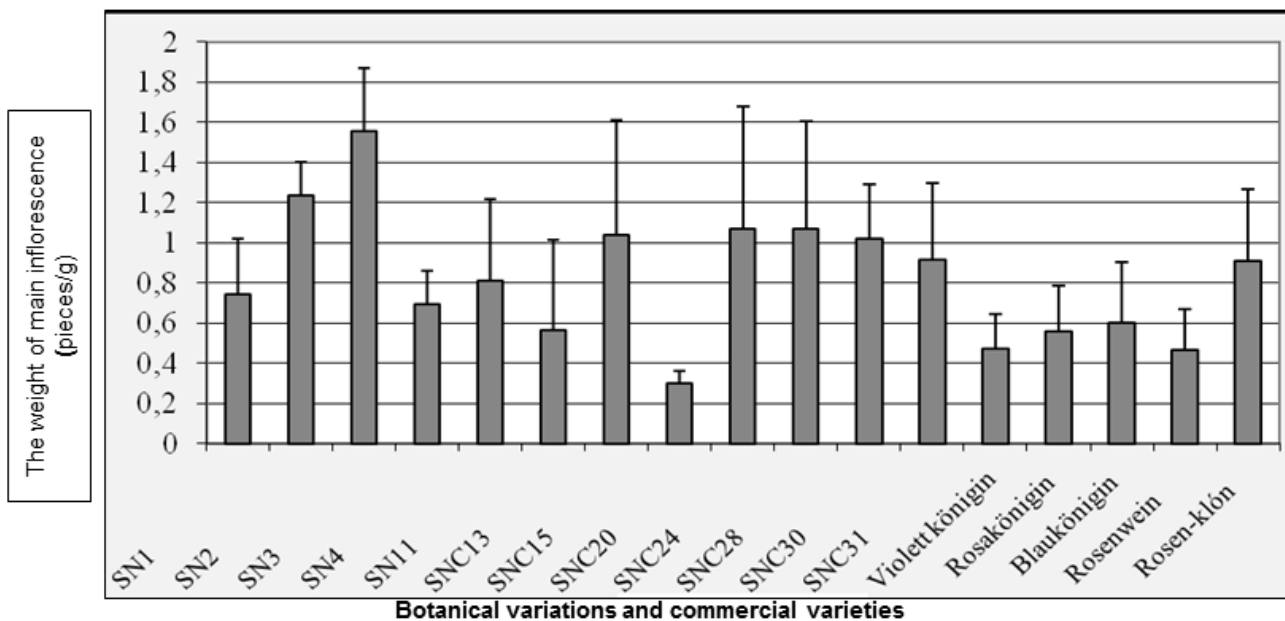


Figure 8. The weight of main inflorescence in *Salvia nemorosa* cultivars and botanical variations in 2014 (pieces/g)

Based on the number of petals located in inflorescence axis (Figure 9.) the value of SN2 and SN3, as well as the SNC31 clone were the highest, the SNC13 and SNC20 variations had less petals. Among the cultivars a conspicuous contrast was observed as the 'Rosenwein' has little petal comparing to its own clone.

3.2. Comparison of ploidy level in botanical woodland sage variations by flow-cytometry

As a control plant we chose *Bellis perennis* species (Figure 10). Figure 11 shows ploidy level of the 12 sage variations /

clones. Based on the nucleus DNA content of daisy we estimated the DNA content of the individuals ranged from 0.87 – 1.37 pg (Table 4). The mean value of this data corresponds to 1.09 pg value found in the Kew's database. There was no detectable difference in ploidy level between the botanical woodland sage variations / clones.

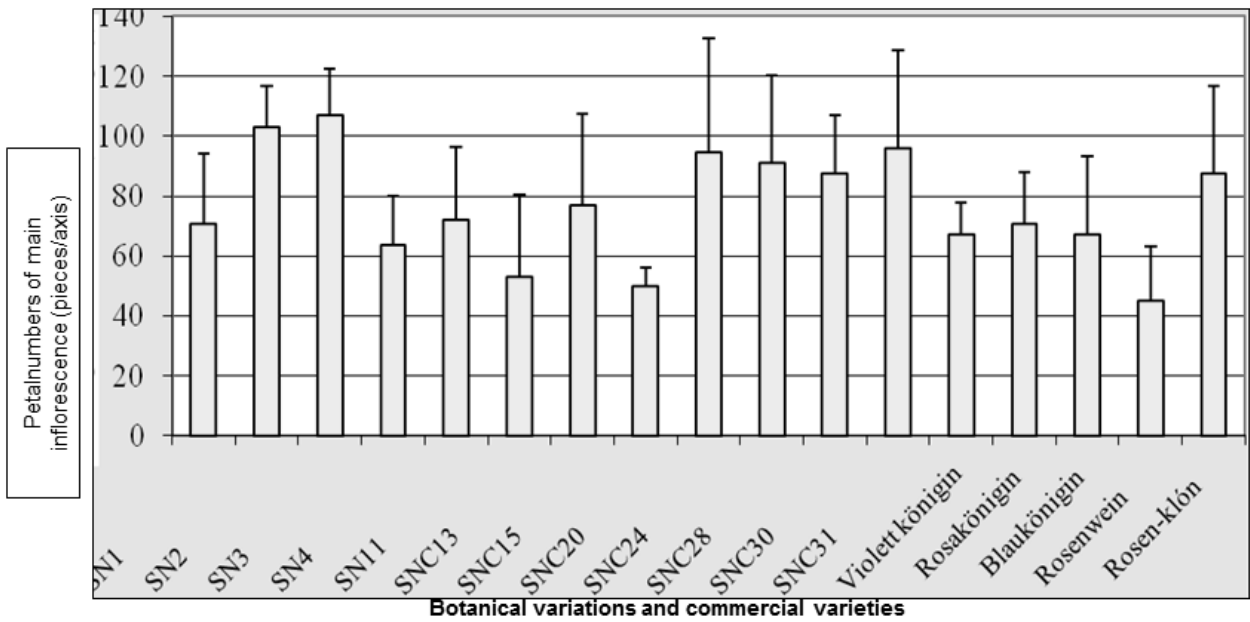


Figure 9. Petal numbers of main inflorescence of woodland sage in 2014 (pieces/axis)

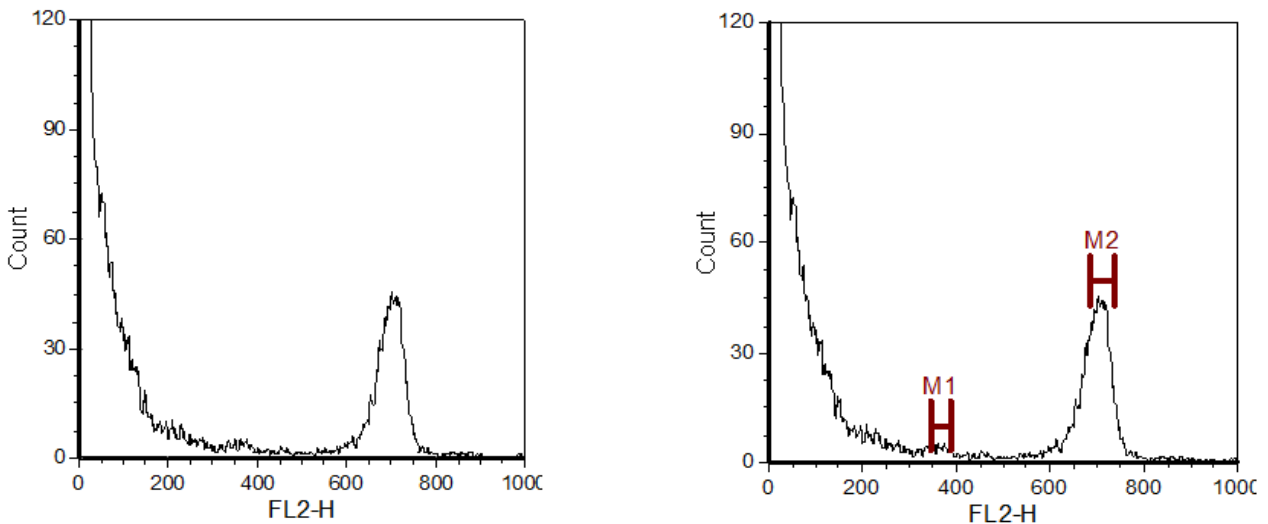


Figure 10. Ploidy level of *Bellis perennis* on *Salvia sp.* setting and its measuring range

Table 4. Estimated DNA content based on the Kew database evaluation

| Sample | SN1 | SN2 | SN3 | SN4 | SN11 | SNC 13 | SNC 15 | SNC 20 | SNC 24 | SNC 28 | SNC 30 | SNC 31 |
|------------|------|------|------|------|------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Value (pg) | 1.15 | 1.37 | 0.99 | 1.08 | 1.15 | 1.17 | 1.03 | 1.13 | 0.87 | 0.89 | 1.20 | 1.12 |

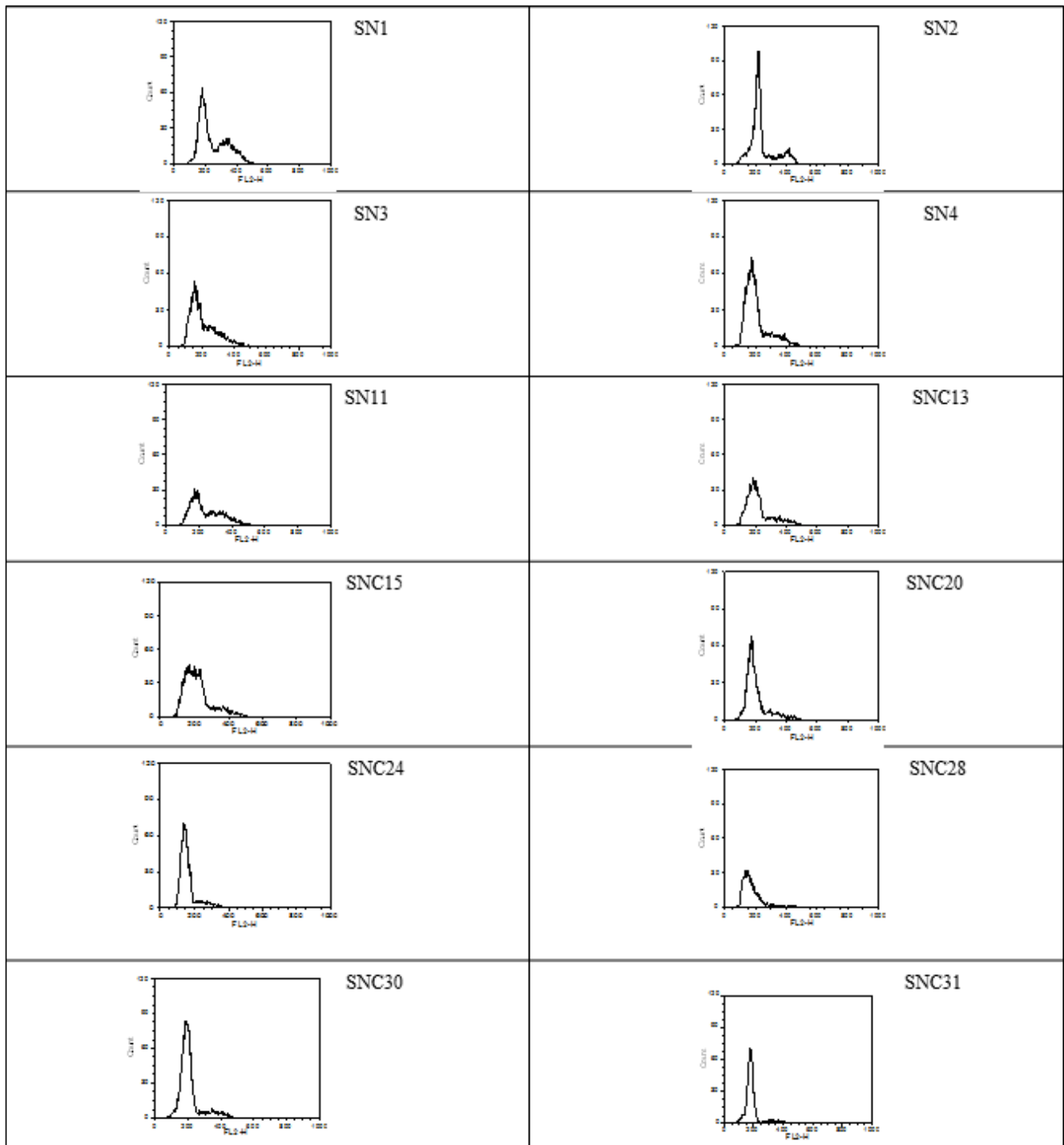


Figure 11. Nuclei fluorescence of leaf samples with PI painted of *Salvia nemorosa* botanical variations/ clones

CONCLUSIONS AND RECOMMENDATIONS

We evaluated the collected woodland sage botanical populations based on morphological parameters. I selected the valuable types having ornamental horticulture value (leaf color, upper and lower lip color, habit, etc.). The vegetatively propagated clones of collected new sage variations can be

planted with each other or together with other drought-tolerant annuals, and perennials in public gardens.

ACKNOWLEDGMENTS

With this written article we want to remember Dr. Zoltán Kováts, who dealt with this plant enthusiastically by searching and preserving its new botanical variations.

Part of this work has been presented at the 15th International Scientific Days of Karoly Robert College (Gyongyos) on March 31, 2016.

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