

# **Cruciferae Newsletter**

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# Acknowledgements

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The current issue of the Cruciferae Newsletter (vol. 35) is published online from the Brassica website (<http://www.brassica.info/info/publications/cruciferae-newsletter.php>). The present issue contains 9 contributions. Members of the editing board would like to acknowledge the authors for the quality of their contributions. For future issues, we would be grateful if all the authors could read and follow carefully the author recommendations before submitting their manuscript, in order to facilitate the editing process. In particular, it is necessary to mention one of the listed topics that is the most relevant to the presented work (see the list at the end of the present issue).

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# Intercropping field mustard (*Brassica rapa* subsp. *oleifera*) with autumn-sown annual legumes and cereals for forage production

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### Introduction

*Brassica rapa* L. is a plant species with a remarkable wide variability of its morphological traits (The Brassica rapa Genome Sequencing Project Consortium 2011). It comprises turnip (*Brassica rapa* L. subsp. *rapa*), a well-known vegetable root crop, several leaf vegetables, such as bok choy (*Brassica rapa* L. subsp. *chinensis* (L.) Hanelt) and napa cabbage (*Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt), and field mustard (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg.), one of the economically important oil crops.

The results of some molecular analyses of field mustard by amplified fragment length polymorphism (AFLP) postulated a possibility that its domestication occurred independently in Europe and East Asia. In both geographic regions, there are the traces of initial breeding attempts, leading to the contemporary genetic diversity of field mustard (Zhao et al. 2005). Today, in the Southeast Europe, field mustard is little known and is often considered a weed and wrongly identified as black mustard (*Brassica nigra* L.).

The main purpose of cultivating field mustard is oil production (Abbasi et al. 2011). Like many other brassica species, it has a numerous additional uses (Hall et al. 2002), such as forage cultivation and green manure. One of the many traits of field mustard desirable in diverse farming systems and crop rotations is prominent earliness, that is, a rather short period from sowing to budding and cutting (Li et al. 2009, Mikić et al. 2014b). Intercropping is widely regarded as growing at least two plant species at the same place in the same time (Willey 1990). Intercropping brassicas, such as fodder kale (*Brassica oleracea* L. var. *viridis* L.), rapeseed (*Brassica napus* L.) and white mustard (*Sinapis alba* L.), with cereals and legumes for forage production proved rather promising in terms of nutritional, yield and quality aspects (Khan et al. 2005, Jamont et al. 2013, Mihailović et al. 2014, Marjanović-Jeromela et al. 2015a, Mikić et al. 2015).

The goal of this preliminary research was to assess the potential of intercropping field mustard with various autumn-sown annual legumes and cereals for forage production.

### Material and methods

A small-plot trial was carried out in 2013 and 2014 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, in the vicinity of Novi Sad, northern Serbia. An experimental field mustard line TR 04 was chosen for intercropping with four autumn-sown annual legumes and four autumn-sown cereals

on the basis of its performance in the previous testing (Marjanović-Jeromela et al. 2015b). The eight intercrop companions of the field mustard line TR 04 were as follow:

1. Common vetch (*Vicia sativa* L.), cultivar NS Tisa;
2. Hairy vetch (*Vicia villosa* Roth), cultivar NS-Viloza;
3. Hungarian vetch (*Vicia pannonica* Crantz), cultivar Panonka;
4. Pea (*Pisum sativum* L.), cultivar NS Krmni;
5. Barley (*Hordeum vulgare* L.), cultivar Nonius;
6. Common wheat (*Triticum aestivum* L. subsp. *aestivum*), cultivar NS 40S;
7. Oat (*Avena sativa* L.), cultivar NS Jadar;
8. Triticale ( $\times$  *Triticosecale* spp.), cultivar Odisej.

In both trial growing seasons, all nine intercrop components were sown during the first decade of October, at the usual sowing rates for each in sole crops, such as 200 viable seeds m<sup>-2</sup> for field mustard (Bilgili et al. 2003) in field mustard, and half-reduced usual sowing rates in the intercrops, with a plot size of 5 m<sup>2</sup> and with three replicates. The sole crops of each species were cut in their own optimum moment: budding in field mustard, full flowering in annual legumes and stage slightly before spikes and racemes appear in cereals.

The intercrops were cut when one of the components was in its optimum moment for cutting, although in most cases they were concurrent in both field mustard and annual legumes or cereals. The fresh forage yield per area unit (t ha<sup>-1</sup>) in all nine sole crops and all eight intercrops was calculated upon the basis of the fresh forage yield per trial plot (kg 5 m<sup>-2</sup>), measured immediately after cutting.

The land equivalent ratio (LER), a parameter for economic justification of intercropping (Mead and Willey 1980), for each intercrop was calculated according to the following formula (Kadžiuilienė et al. 2011):

$$\text{LER} = \text{FM}_{\text{IC}} / \text{ALC}_{\text{SC}} + \text{FM}_{\text{IC}} / \text{ALC}_{\text{SC}},$$

where FM<sub>IC</sub> is the fresh forage yield of field mustard in an intercrop with annual legumes or cereals, FM<sub>SC</sub> is the fresh forage yield of field mustard in its sole crop, ALC<sub>IC</sub> is the fresh forage yield of an annual legume or cereal component in an intercrop and ALC<sub>SC</sub> is the fresh forage yield of an annual legume or cereal component in its sole crop.

The results of the trial were processed by means of analysis of variance (ANOVA) and the Least Significant Difference (LSD) test.

## Results and Discussion

Significant differences were determined among the two-year average values of the fresh forage yield in the fresh forage yield of sole crops, the fresh forage yield of single components, the total fresh forage yield and its LER (Tables 1 and 2).

In the sole crops, field mustard had much significantly higher fresh forage yield (75.1 t ha<sup>-1</sup>) than the annual legumes and the cereals (Table 1). It had slightly higher fresh forage yield than fodder kale in previous research, with 67.5 t ha<sup>-1</sup>, as well as than autumn- and spring sown rapeseed and white mustard (Mihailović et al. 2008). The highest values of the fresh forage yield among the annual legumes and the cereals were 50.1 t ha<sup>-1</sup> in pea and 50.5 t ha<sup>-1</sup> in triticale, with both achieving better performance than in the trial with intercropping pea with cereals in the same agroecological conditions (Mihailović et al. 2011).

**Table 1. Average values of fresh forage yield (t ha<sup>-1</sup>) in the sole crops of field mustard and autumn-sown annual legumes and cereals in the trial at Rimski Šančevi for 2013 and 2014**

Crop	Species	Fresh forage yield
Brassica	Field mustard	75.1
Annual legume	Common vetch	40.4
	Hairy vetch	42.5
	Hungarian vetch	35.7
	Pea	50.1
	Average	42.2
Cereal	Barley	49.2
	Common wheat	40.4
	Oat	40.1
	Triticale	50.5
	Average	45.1
<i>LSD</i> <sub>0.05</sub>		7.7

In the intercrops of field mustard and the autumn-sown annual legumes (Table 2), field mustard had the greatest individual contribution to the total fresh forage yield in the mixture with pea (33.5 t ha<sup>-1</sup>). On the other hand, hairy vetch proved to be the most aggressive among the annual legumes, at the same time achieving 38.0 t ha<sup>-1</sup> and leaving field mustard with 16.5 t ha<sup>-1</sup> of fresh forage. Hairy vetch was also more dominant in a trial with intercropping with fodder kale and rapeseed, with 6.1 t ha<sup>-1</sup> and 6.6 t ha<sup>-1</sup> of forage dry matter (Ćupina et al. 2013).

**Table 2. Average values of fresh forage yield (t ha<sup>-1</sup>) its land equivalent ratio (LER) in the intercrops of field mustard with autumn-sown annual legumes and cereals in the trial at Rimski Šančevi for 2013 and 2014**

Intercrop	Field mustard fresh forage yield	Annual legume / cereal fresh forage yield	Total fresh forage yield	LER
Field mustard + common vetch	23.1	36.3	59.4	1.21
Field mustard + hairy vetch	16.5	38.0	54.5	1.11
Field mustard + Hungarian vetch	20.1	35.1	55.2	1.25
Field mustard + pea	33.5	36.7	70.2	1.18
Field mustard + legumes average	23.3	36.5	59.8	1.19
Field mustard + barley	24.6	33.5	58.1	0.95
Field mustard + common wheat	28.7	23.8	52.5	1.03
Field mustard + oat	27.6	25.1	52.7	1.02
Field mustard + triticale	25.7	27.5	53.2	0.88
Field mustard + cereals average	26.7	27.5	54.1	0.97
<i>LSD</i> <sub>0.05</sub>	2.3	3.9	2.0	0.05

In average, field mustard had greater contribution to the total fresh forage yield when intercropped with cereals (26.7 t ha<sup>-1</sup>) than with annual legumes (Table 2). It ranged from 24.6 t ha<sup>-1</sup> in the intercrop with barley to 28.7 t ha<sup>-1</sup> in the intercrop with common wheat. Barley demonstrates a similar ability to be highly competitive with other crops, such as annual legumes (Mihailović et al. 2004).

The two-year average fresh forage yield ranged from 52.5 t ha<sup>-1</sup> in the intercrop of field mustard with common wheat to 70.2 t ha<sup>-1</sup> in the intercrop of field mustard and pea (Table 2). The intercrops of field mustard with annual legumes had higher two-year average fresh forage yield (59.8 t ha<sup>-1</sup>) than the intercrops with cereals (54.1 t ha<sup>-1</sup>), what is congruent with the previously conducted testing (Mikić et al. 2014a).

All four intercrops of field mustard and annual legumes proved economically justified, with the two-year average LER values ranging between 1.11 in the intercrop with hairy vetch and 1.25 in the intercrop with Hungarian vetch. These values were significantly lower in the intercrops with cereals, with those with barley and triticale lower than 1 (Table 2).

## Conclusions

There is a solid basis to deem intercropping field mustard with autumn-sown legumes and cereals reliable, in terms of both fresh forage yield and the economic aspect of such production, especially significant in feeding milk cows. The future research efforts should be focused on the quality aspects and stress-related issues.

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## References

- Abbasi BH, Khan M, Guo B, Bokhari SA, Khan MA (2011) Efficient regeneration and antioxidative enzyme activities in *Brassica rapa* var. turnip. *Plant Cell Tissue Organ Cult* 105:337-344
- Bilgili U, Sincik M, Uzun A, Acikgoz E (2003) The influence of row spacing and seeding rate on seed yield and yield components of forage turnip (*Brassica rapa* L.). *J Agron Crop Sci* 189:250-254
- Ćupina B, Mikić A, Marjanović-Jeromela A, Krstić Đ, Antanasović S, Erić P, Vasiljević S, Mihailović V (2013) Intercropping autumn-grown brassicas with legumes for forage production. *Crucif Newsl* 32:17-19
- Hall JC, Sytsma KJ, Iltis HH (2002) Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *Am J Bot* 89:1826-1842
- Jamont M, Piva G, Fustec J (2013) Sharing N resources in the early growth of rapeseed intercropped with faba bean: does N transfer matter? *Plant Soil* 371:641-653
- Kadžiulienė Ž, Šarūnaitė L, Deveikytė I (2011) Effect of pea and spring cereals intercropping on grain yield and crude protein content. *Ratar Povrt* 48:183-188
- Khan M, Khan RU, Wahab A, Rashid A (2005) Yield and yield components of wheat as influenced by intercropping of chickpea, lentil and rapeseed in different proportions. *Pak J Agric Sci* 42:3-4
- Li F, Kitashiba H, Inaba K, Nishio T (2009) A *Brassica rapa* linkage map of EST-based SNP markers for identification of candidate genes controlling flowering time and leaf morphological traits. *DNA Res* 16:311-323
- Marjanović-Jeromela A, Mikić A, Mihailović V, Aćin V, Kondić-Špika A, Hristov N, Terzić S (2015a) Forage dry matter crude protein yield in the intercrops of spring-sown brassicas with cereals. *Crucif Newsl* 34:17-19
- Marjanović-Jeromela A, Mikić A, Terzić S, Marinković R, Mihailović V (2015b) Fresh forage yield and its components in turnip rape (*Brassica rapa* subsp. *oleifera*). *Crucif Newsl* 34:23-25
- Mead R, Willey RW (1980) The concept of a 'land equivalent ratio' and advantages in yields from intercropping. *Exp Agric* 16:217-228
- Mihailović V, Erić P, Marinković R, Ćupina B, Marjanović-Jeromela A, Mikić A, Krstić Đ, Červenski J (2008) Potential of some brassica species as forage crops. *Crucif Newsl* 27:39-40
- Mihailović V, Mikić A, Kobiljski B, Ćupina B, Antanasović S, Krstić Đ, Katanski S (2011) Intercropping pea with eight cereals for forage production. *Pisum Genet* 43:33-35
- Mihailović V, Mikić A, Ćupina B, Marjanović-Jeromela A, Marinković R, Živanov D, Kondić-Špika A, Hristov N, Denčić S (2014a) Intercropping spring-sown brassicas with cereals for forage production. *Crucif Newsl* 33:16-18
- Mikić A, Mihailović V, Marjanović-Jeromela A, Terzić S (2014b) Certain aspects of breeding forage brassicas. In: Sokolović D, Huyghe C, Radović J (eds) *Quantitative Traits Breeding for Multifunctional Grasslands and Turf*. Springer Science+Business Media, Dordrecht, the Netherlands, 163-166
- Mikić A, Ćupina B, Rubiales D, Mihailović V, Šarūnaitė L, Fustec J, Antanasović S, Krstić Đ, Bedoussac L, Zorić L, Đorđević V, Perić V, Srebrić M (2015) Models, developments, and perspectives of mutual legume intercropping. *Adv Agron* 130:337-419
- The Brassica rapa Genome Sequencing Project Consortium (2011) The genome of the mesopolyploid crop species *Brassica rapa*. *Nat Genet* 43:1035-1039
- Willey RW (1990) Resource use in intercropping systems. *Agric Water Manag* 17:215-231.
- Zhao J, Wang X, Deng B, Lou P, Wu J, Sun R, Xu Z, Vromans J, Koornneef M, Bonnema G (2005) Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. *Theor Appl Genet* 110:1301-1314

## Intercropping autumn-sown brassicas with cereals for green manure

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### Introduction

Both brassicas and cereals are among the most traditional crops in the Southeast Europe. Apart from their individual main purposes, they are often cultivated for forage, such as fodder kale (*Brassica oleracea* L. var. *viridis* L.), rapeseed (*Brassica napus* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), triticale ( $\times$  *Triticosecale* spp.) or common wheat (*Triticum aestivum* L. subsp. *aestivum*) (Mikić et al. 2014b).

It seems that growing more than one crop at the same place and at the same time together, usually referred to as *intercropping*, is one of the oldest agricultural practices in the world (Abbo et al. 2010). One of its most traditional ways includes the mixtures of primarily cereals and annual legumes, such as such as pea (*Pisum sativum* L.) or vetches (*Vicia* spp.) (Mihailović et al. 2004). Although still less explored, intercropping cereals with brassicas has propelled during the last decade (Khan et al. 2005) and demonstrated various benefits for the yield components, yield *per se* and yield quality in both components (Mikić et al. 2014a, Mihailović et al. 2015).

This study was targeting the potential of intercropping autumn-sown brassicas with cereals for green manure and thus the value of their environment-friendly use.

### Materials and Methods

A small-plot trial was conducted during the growing seasons of 2012/2013 and 2013/2014 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, in the vicinity of Novi Sad. It comprised eight intercrops of two autumn-sown brassicas, namely fodder kale and rapeseed, with four autumn-sown cereals, namely oat, barley, triticale and common wheat, as well as six sole crops.

In both trial years, all eight intercrops and six sole crops were sown in the last decade of September, at a half-reduced sowing rate in the intercrops. In both sole crops and intercrops, two brassicas were cut in full budding and the very beginning of bloom, while four cereals were cut several days before spikes or racemes were to appear.

The aboveground biomass nitrogen yield ( $\text{kg ha}^{-1}$ ) in all the sole crops and intercrops was calculated on the basis of the values of forage dry matter yield ( $\text{kg ha}^{-1}$ ) and forage dry matter nitrogen content ( $\text{g kg}^{-1}$ ), determined by the standardised method by Kjeldahl.

The average values of the land equivalent ratio (LER) for aboveground biomass nitrogen yield were calculated

according to the following formula (Mihailović et al. 2011):

$$LER = B_{IC} / B_{SC} + C_{IC} / C_{SC},$$

where  $B_{IC}$  is the aboveground biomass nitrogen yield of a brassica in an intercrop with a cereal,  $B_{SC}$  is the aboveground biomass nitrogen yield of a brassica in its sole crop,  $C_{IC}$  is the aboveground biomass nitrogen yield of a cereal in an intercrop and  $C_{SC}$  is the aboveground biomass nitrogen yield of a cereal in its sole crop. The obtained results were processed by analysis of variance (ANOVA) with the Least Significant Difference (LSD) test.

## Results and Discussion

There were significant differences in two-year average values of aboveground biomass nitrogen yield and their LER in sole crops and intercrops of autumn-sown forage brassicas and cereals (Table 1).

Overall, the two-year average aboveground biomass nitrogen yield in the sole crops of brassicas was 209 kg ha<sup>-1</sup>, while in the sole crops of cereals it was 268 kg ha<sup>-1</sup>. The two-year average aboveground biomass nitrogen yield among species varied between 205 kg ha<sup>-1</sup> in rapeseed and 296 kg ha<sup>-1</sup> in oat. It is notable that the sole crops of both autumn-sown brassicas produced significantly higher aboveground biomass nitrogen yield than ten fodder kale cultivars and lines in the same agroecological conditions, with 183 kg ha<sup>-1</sup> as their individual maximum (Ćupina et al. 2010).

Considering the intercrops of autumn-sown forage brassicas and cereals, the average two-year values of total aboveground biomass nitrogen yield ranged from 274 kg ha<sup>-1</sup> in the intercrop of fodder kale and barley to 303 kg ha<sup>-1</sup> in the intercrop of rapeseed and oat. Among brassicas, the highest two-year average individual contribution to the total aboveground biomass nitrogen yield was in fodder kale intercropped with triticale (98 kg ha<sup>-1</sup>), while the lowest two-year average individual contribution to the total aboveground biomass nitrogen yield was in rapeseed intercropped with barley (84 kg ha<sup>-1</sup>). The variation of the aboveground biomass nitrogen yield in the intercrops of autumn-sown brassicas and cereals was much narrower than in a trial with the intercrops of autumn-sown brassicas and annual legumes, with amplitude of 157 kg ha<sup>-1</sup> (Mikić et al. 2015). All the intercrops of autumn-sown brassicas and cereals had the average two-year values of LER higher than 1, with those involving common wheat (1.33 and 1.34) as the most economically reliable.

**Table 1. Average values of aboveground biomass nitrogen yield (kg ha<sup>-1</sup>) in the sole crops of autumn-sown brassicas and cereals in the trial at Rimski Šančevi for 2012/2013 and 2013/2014**

Sole crop / Intercrop	Brasica aboveground biomass nitrogen yield	Cereal aboveground biomass nitrogen yield	Total aboveground biomass nitrogen yield	LER
Fodder kale	213	-	213	-
Rapeseed	205	-	205	-
Brassicas average	209	-	209	-
Barley	-	247	247	-
Common wheat	-	258	258	-
Oat	-	296	296	-
Triticale	-	269	269	-
Cereals average	-	268	268	-
Fodder kale + barley	88	186	274	1.13
Fodder kale + common wheat	90	196	286	1.34
Fodder kale + oat	92	206	298	1.10
Fodder kale + triticale	98	201	299	1.19
Fodder kale + cereal average	92	197	289	1.19
Rapeseed + barley	84	190	280	1.15
Rapeseed + common wheat	87	195	282	1.33
Rapeseed + oat	93	210	303	1.14
Rapeseed + triticale	89	202	291	1.17
Rapeseed + cereal average	88	199	288	1.20
LSD <sub>0.05</sub>	19	16	18	0.04

## Conclusions

Intercropping autumn-sown forage brassicas with cereals proved to have a desirable potential for an economically reliable production of high and stable yield of aboveground biomass nitrogen and thus offer a beneficial ecological services to modern cropping systems and enriching them with species diversity. A thorough analysis of degrading each intercrop in the soil and its effect on the nutrients variability, quantity and mobility would be one of the future steps with the highest priority.

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## References

- Abbo S, Lev-Yadun S, Gopher A (2010) Agricultural origins: Centers and noncenters; A Near Eastern reappraisal. *Crit Rev Plant Sci* 29:317-328
- Ćupina B, Krstić Đ, Antanasović S, Erić P, Manojlović M, Čabilovski R, Mikić A (2010) Potential of fodder kale (*Brassica oleracea* L. var. *viridis* L.) as a green manure crop. *Crucif Newsl* 29:10-11
- Khan M, Khan RU, Wahab A, Rashid A (2005) Yield and yield components of wheat as influenced by intercropping of chickpea, lentil and rapeseed in different proportions. *Pak J Agric Sci* 42:3-4
- Mihailović V, Erić P, Mikić A (2004) Growing peas and vetches for forage in Serbia and Montenegro. *Grassl Sci Eur* 9:457-459
- Mihailović V, Mikić A, Kobiljski B, Ćupina B, Antanasović S, Krstić Đ, Katanski S (2011) Intercropping pea with eight cereals for forage production. *Pisum Genet* 43:33-35
- Mihailović V, Mikić A, Marjanović-Jeromela A, Mirosavljević M, Hristov N, Kondić-Špika A, Terzić S (2015) Forage dry matter crude protein yield in the intercrops of autumn-sown brassicas with cereals. *Crucif Newsl* 34:20-22
- Mikić A, Ćupina B, Mihailović V, Marjanović-Jeromela A, Terzić S, Antanasović S, Katanski S, Živanov D, Aćin V (2014a) Intercropping autumn-sown brassicas with cereals for forage production. *Crucif Newsl* 33:22-24
- Mikić A, Mihailović V, Marjanović-Jeromela A, Terzić S (2014b) Certain aspects of breeding forage brassicas. Proceedings, 30th EUCARPIA Fodder Crops and Amenity Grasses Section Meeting *Quantitative Traits Breeding for Multifunctional Grasslands and Turf*, Vrnjačka Banja, Serbia, 12-16 May 2013, 163-166
- Mikić A, Antanasović S, Ćupina B, Marjanović-Jeromela A, Erić P, Jakšić S, Terzić S (2015) Intercropping autumn-sown brassicas with annual legumes for green manure. *Cruciferae Newsletter*, 34, 11-13

# Intercropping spring-sown brassicas with cereals for green manure

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### Introduction

Brassica and cereal crops have been cultivated in Southeast Europe since Neolithic, as one of the major segments of the so-called “agricultural revolution”, having commenced in the Near East (Zohary et al. 2012). The Balkan Peninsula was one of its main routes leading to the continent’s centre and has remained oriented towards growing these crops until today. In many regions, spring-sown cultivars of both brassicas, such as rapeseed (*Brassica napus* L.) and white mustard (*Sinapis alba* L.), and cereals, like oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and common wheat (*Triticum aestivum* L. subsp. *aestivum*) are used for forage production, either as sole crops or in mixtures mostly with annual legumes, such as pea (*Pisum sativum* L.) or common vetch (*Vicia sativa* L.) (Ćupina et al. 2011, Ćupina et al. 2014).

Intercropping, most often referring to sowing and cultivating two or more domesticated species at the same place and at the same time together, is one of the most ancient attested farming designs (Hauggaard-Nielsen et al. 2011). Mixtures of brassicas and legumes proved to be beneficial to both components, especially for the first one, due to an enhanced supply with nitrogen (Cortés-Mora et al. 2010). The agronomic performance of the intercrops of various spring-sown brassicas and cereals has remained rather scarcely examined, although it could provide diverse agricultural practices in contrasting temperate environments with a number of advantages (Mihailović et al. 2014).

The goal of this study was to assess the possibility of intercropping spring-sown brassicas with cereals for green manure, thus examining its suitability for ecological services.

### Materials and Methods

A small-plot trial was established during the growing seasons of 2013 and 2014 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, near the city of Novi Sad. It encompassed six intercrops of two spring-sown brassicas, rapeseed and white mustard, with three spring-sown cereals, oat, barley and common wheat, as well as sole crops of all five examined species.

In both trial seasons, all six intercrops and five sole crops were sown in the first decade of March, with a double-reduced sowing rate in the intercrops, providing no more expensive sowing costs in a potential

wide-scale production. In both sole crops and intercrops, two brassicas were cut in the stages of full budding and the very commencement of flowering, while three cereals were cut at the onset of appearing of spikes and racemes.

In all the sole crops and intercrops, the aboveground biomass nitrogen yield ( $\text{kg ha}^{-1}$ ) calculation was based upon the values of forage dry matter yield ( $\text{kg ha}^{-1}$ ) and forage dry matter nitrogen content ( $\text{g kg}^{-1}$ ), as determined by the Kjeldahl method.

The land equivalent ratio (LER) values for aboveground biomass nitrogen yield were calculated after the following formula (Mikić et al. 2015):

$$\text{LER} = \text{SB}_{\text{IC}} / \text{SB}_{\text{SC}} + \text{SC}_{\text{IC}} / \text{SC}_{\text{SC}},$$

where  $\text{SB}_{\text{IC}}$  is the aboveground biomass nitrogen yield of a brassica in an intercrop with a cereal,  $\text{SB}_{\text{SC}}$  is the aboveground biomass nitrogen yield of a brassica in its sole crop,  $\text{SC}_{\text{IC}}$  is the aboveground biomass nitrogen yield of a cereal in an intercrop and  $\text{SC}_{\text{SC}}$  is the aboveground biomass nitrogen yield of a cereal in its sole crop. The results were processed by analysis of variance (ANOVA) with the Least Significant Difference (LSD) test.

## Results and Discussion

Among the two-year average values of aboveground biomass nitrogen yield and their LER in sole crops and intercrops of spring-sown forage brassicas and cereals, significant differences at a level of 0.05 were determined (Table 1).

In the sole crops of the spring-sown brassicas, the two-year average aboveground biomass nitrogen yield was  $196 \text{ kg ha}^{-1}$ , while in the sole crops of the spring-sown cereals it was  $210 \text{ kg ha}^{-1}$ . Among the five individual examined species, the two-year average aboveground biomass nitrogen yield ranged from  $186 \text{ kg ha}^{-1}$  in rapeseed to  $222 \text{ kg ha}^{-1}$  in oat. The sole crops of both rapeseed and white mustard had much higher aboveground biomass nitrogen yield in comparison to the results of two preliminary trials in the same environment, with the maximum values of  $132 \text{ kg ha}^{-1}$  in rapeseed (Krstić et al. 2012) and  $138 \text{ kg ha}^{-1}$  in white mustard (Krstić et al. 2010).

Regarding the average two-year values of total aboveground biomass nitrogen yield in the intercrops of spring-sown forage brassicas and cereals, it varied between  $194 \text{ kg ha}^{-1}$  in the intercrop of white mustard and barley to  $266 \text{ kg ha}^{-1}$  in the intercrop of white mustard and oat. Among brassicas, rapeseed had the highest two-year average individual contribution to the total aboveground biomass nitrogen yield when intercropped with common wheat ( $113 \text{ kg ha}^{-1}$ ), while white mustard had the lowest two-year average individual contribution to the total aboveground biomass nitrogen yield when intercropped with oat ( $76 \text{ kg ha}^{-1}$ ). The aboveground biomass nitrogen yield in the intercrops of spring-sown brassicas and cereals had a significantly smaller variation in comparison to a trial with the intercrops of spring-sown brassicas and annual legumes, with amplitude of  $174 \text{ kg ha}^{-1}$  (Marjanović Jeromela et al. 2015). All the intercrops of autumn-sown brassicas and cereals had the average two-year values of LER higher than 1, with rapeseed and common wheat being significantly the most economically reliable (1.24).

## Conclusions

Producing mixtures of various spring-sown forage brassicas with cereals may be an additional source of environment-friendly increasing of soil fertility in temperate and other regions, providing high and stable aboveground biomass nitrogen yield for different contemporary cropping systems and contributing to their species diversity, and with future analyses of degrading incorporated aboveground biomass in the soil and its effect on the nutrients behaviour as highly desirable.

**Table 1. Average values of aboveground biomass nitrogen yield (kg ha<sup>-1</sup>) in the sole crops of spring-sown brassicas and cereals in the trial at Rimski Šančevi for 2013 and 2014**

Sole crop / Intercrop	Brasica aboveground biomass nitrogen yield	Cereal aboveground biomass nitrogen yield	Total aboveground biomass nitrogen yield	LER
Rapeseed	186	-	-	-
White mustard	206	-	-	-
Brassicas average	196	-	-	-
Barley	-	200	-	-
Common wheat	-	209	-	-
Oat	-	222	-	-
Cereals average	-	210	-	-
Rapeseed + barley	107	135	242	1.16
Rapeseed + common wheat	113	120	233	1.24
Rapeseed + oat	101	130	230	1.13
Rapeseed + cereal average	107	128	235	1.18
White mustard + barley	99	122	194	1.03
White mustard + common wheat	82	133	235	1.10
White mustard + oat	76	180	266	1.15
White mustard + cereal average	86	145	232	1.09
LSD <sub>0.05</sub>	22	18	21	0.06

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## References

- Cortés-Mora FA, Piva G, Jamont M, Fustec J (2010) Niche separation and nitrogen transfer in Brassica-legume intercrops. *Ratar Povrt* 47:581-586
- Ćupina B, Manojlović M, Krstić Đ, Čabilovski R, Mikić A, Ignjatović-Ćupina A, Erić P (2011) Effect of winter cover crops on the dynamics of soil mineral nitrogen and yield and quality of Sudan grass (*Sorghum bicolor* (L.) Moench). *Aust J Crop Sci* 5:839-845
- Ćupina B, Mikić A, Marjanović-Jeromela A, Živanov D, Krstić Đ, Antanasović S, Terzić S, Erić P (2014) Forage dry matter crude protein yield in the intercrops of spring-sown brassicas with legumes. *Crucif Newsl* 33:13-15
- Hauggaard-Nielsen H, Peoples MB, Jensen ES (2011) Faba bean in cropping systems. *Grain Legum* 56:32-33
- Krstić Đ, Ćupina B, Antanasović S, Erić P, Čabilovski R, Manojlović M, Mikić A (2010) Potential of white mustard (*Sinapis alba* L. subsp. *alba*) as a green manure crop. *Crucif Newsl* 29:12-13
- Krstić Đ, Ćupina B, Antanasović S, Čabilovski R, Manojlović M, Marjanović-Jeromela A, Mikić A (2012) Potential of spring-sown rapeseed (*Brassica napus*) as a green manure crop. *Crucif Newsl* 31:29-30
- Marjanović-Jeromela A, Mikić A, Ćupina B, Antanasović S, Erić P, Terzić S, Miladinović D (2015) Intercropping spring-sown brassicas with annual legumes for green manure. *Crucif Newsl* 34:8-10
- Mihailović V, Mikić A, Ćupina B, Marjanović-Jeromela A, Marinković R, Živanov D, Kondić-Špika A, Hristov N, Denčić S (2014) Intercropping spring-sown brassicas with cereals for forage production. *Crucif Newsl* 33:16-18
- Mikić A, Ćupina B, Rubiales D, Mihailović V, Šarūnaitė L, Fustec J, Antanasović S, Krstić Đ, Bedoussac L, Zorić L, Đorđević V, Perić V, Srebić M (2015) Models, developments, and perspectives of mutual legume intercropping. *Adv Agron* 130:337-419
- Zohary D, Hopf M, Weiss E (2012) Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press, Oxford

# Evaluation of tropical cauliflower (*Brassica oleracea* L. var. *botrytis*) for South India

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### Abstract

The effect of sowing date and varieties on the growth and yield of cauliflower was studied under the Agro climatic conditions of South India. Four sowing dates and twelve varieties were used in this study. Analysis of variance revealed significant difference among sowing dates, varieties and their interactions for all the characters studied. Among the sowing dates, November 1<sup>st</sup> sowing recorded better vegetative, leaf size curd and yield characters. Among the varieties, NS 60N was the highest yielder (454.02 g) based on yield characters. Curd depth, curd diameter and curd size index were also highest for NS 60N followed by G 45. Earliest among the varieties was Himshort followed by NS 60N and the late varieties were Pusa Sharad and Pusa Hybrid-2. Himpriya-60 exhibited highest plant height, leaves per plant, gross plant weight, leaf length, leaf breadth and leaf size. Interaction effects were significant and yield characters were best for NS 60N sown on November 1<sup>st</sup>. The study identified two high yielding varieties namely NS 60N and G 45 as promising and November 1<sup>st</sup> sowing as the best sowing time for cultivation in South India.

**Keywords:** Cauliflower, Date of Sowing, Varieties, Interaction

### Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is a biannual and herbaceous vegetable crop belonging to the family Brassicaceae. Cauliflower is grown for its white tender compact curd formed by the shortened flower parts. Curd is a hypertrophied pre floral meristamatic growth, which terminates main stem of the plant. Temperature plays crucial role in curd formation of cauliflower. Apart from varieties, time of planting is another key factor which determines the productivity. In general, the cooler months of October - January is ideal for cauliflower cultivation in South India. Since the varieties are very specific in its temperature requirement for curding, identification of the most suitable time of sowing for a particular variety will definitely help in increasing productivity.

### Materials and Methods

The experiment was conducted at the Department of Olericulture, College of Agriculture, Vellayani, during the period October 2012 to March 2013. Split plot design was adopted for the layout of the experiment with four sowing dates as main plot treatments and twelve varieties as sub plot treatments. The mainplot treatments viz,

October 1<sup>st</sup>, October 15<sup>th</sup>, November 1<sup>st</sup>, November 15<sup>th</sup> sowing dates and subplot treatments viz, Pusa Meghna, Pusa Sharad, Pusa Paushja, Pusa Hybrid 2, Pusa Shukti, NS 60 N, Himshort, Himlatha, Himpriya-60, Indam 2435, G 45, White Snow. The seedlings were raised in protrays and one month old seedlings were transplanted into the main field at a spacing of 60 x 60 cm. All cultural operations like weeding, fertilizer application, irrigation, earthing up and spraying of pesticides were done as per the recommendations (KAU, 2011). Observations were recorded on five randomly selected competitive plants per replication for each entry on sixteen traits, viz., plant height (cm), leaves per plant, gross plant weight (kg), leaf size (cm<sup>2</sup>), days to curd initiation, days to curd harvest, curd depth (cm), curd diameter (cm), curd size index (cm<sup>2</sup>), stalk length (cm), net curd weight (g), gross curd weight (g), harvest index, protein (%), vitamin C (mg) and vitamin A (IU) contents.

## Results and Discussion

The response of tropical cauliflower to different sowing dates, varieties and their interaction revealed significant differences with respect to vegetative, curd and yield characters. In the present study, November 1<sup>st</sup> sowing resulted in maximum plant height, leaves per plant, gross plant weight, leaf length, leaf breadth and leaf size. Similarly, influence of sowing dates on different vegetative characters like leaves per plant, leaf area index, plant weight were reported by Ajithkumar (2005), Kaur *et al.* (2007) and Din *et al.* (2007). The better plant growth of November 1<sup>st</sup> sowing might be due to conducive climatic conditions which in turn resulted in high dry matter accumulation. Among the varieties Himpriya 60 excelled other varieties in overall performance with respect to all the vegetative characters like plant height, leaves per plant, gross plant weight, leaf length, leaf breadth and leaf size whereas, Himshort recorded the least values. Interaction effects for dates of sowing and varieties were also significant for all vegetative characters. Cumulative effect of best sowing date and variety for vegetative characters were reflected in their interaction too.

Earliness in curd initiation, maturity and harvest are preferred characters in cauliflower since the duration of winter is too short in the plains of Southern India. The plants will remain in their vegetative phase till the advent of favorable temperature for curd initiation. A temperature of 20-24°C is optimum for curding in early cultivars of cauliflower (Nieuwhof, 1969). In the present study, the days to curd initiation, maturity and harvest were significantly altered by sowing dates, varieties and their interaction. November 15<sup>th</sup> sowing resulted in early curd initiation (47.14 days), since they received high temperature during early growth stage. These findings are in conformity with the findings of Pradeepkumar *et al.* (2002) who reported a similar range for days to maturity in cauliflower. Among the varieties, Himshort was the earliest followed by NS 60N and the late ones were Pusa Sharad and Pusa Hybrid 2 which are mid season varieties. October 1<sup>st</sup> sowing of Himshort resulted in earliest curd initiation (37.22 days) and the interaction between sowing dates and varieties for days to curd initiation and maturity were earlier reported by Yadav *et al.* (1995), Callens *et al.* (2000) and Pradeepkumar *et al.* (2002).

Among the different sowing dates, November 1<sup>st</sup> sowing recorded highest curd depth, curd diameter and curd size index. Such result may be attributed to the fact that plants in November 1<sup>st</sup> sowing got better opportunity to develop vegetatively, since they received favorable weather. Adequate vegetative growth and carbohydrate accumulation contributes a lot in the development of economic part in cauliflower, hence vigorous plants ultimately led to larger curd size. In contrast, those sowing dates having inadequate vegetative growth resulted into small curds. Curd characters like curd depth, curd diameter and curd size index were highest for NS 60N and lowest for Pusa Sharad and Pusa Hybrid 2. Among interaction effects, maximum curd depth, curd diameter and curd size index were observed in October 1<sup>st</sup> sowing of Pusa Hybrid 2 and November 1<sup>st</sup> sowing of NS 60N.

Yield is the most important factor in any crop production. In cauliflower, curd is the economic part and the net curd weight was found to be influenced by different sowing dates. It was highest for November 1<sup>st</sup> sowing

(361.69 g) followed by that of October 1<sup>st</sup> (336.57 g) hence curd weight was greatly influenced by temperature. It was clear from the result that in South India, a difference of 15 days in sowing resulted in remarkable reduction in curd yield. Among varieties, the best performers with respect to net curd weight, gross curd weight and yield per plot were NS 60 N followed by G 45 whereas Pusa Sharad and Pusa Hybrid 2 were poor yielders. The present result is in accordance with the finding Narayanankutty (2012) who identified NS 60N as the suitable variety for the warm humid tropics of South India. November 1<sup>st</sup> sowing of NS 60 resulted in highest net curd weight (629.33 g) and complete curding was observed for Pusa Meghna, NS 60N, Himshort, Himpriya 60 and G 45 in all the four sowing dates.

Quality characters are as important as yield in food crops especially vegetables. But in most of the cases quality is negatively correlated with yield. In the present study, it was observed that protein, Vitamin A and vitamin C content were highly influenced by genotype rather than environment. Among the varieties high protein and Vitamin A content was observed in Pusa Hybrid 2 and Pusa Meghna respectively. No significant difference was observed for vitamin C content among varieties. Pusa hybrid 2 and Pusa Meghna sown on November 15 had high protein and Vitamin A content respectively. For Vitamin C content no significant difference was observed between the sowing dates and varieties.

The study identified two high yielding varieties namely NS 60N and G 45 as promising and November 1<sup>st</sup> sowing as the best sowing time for cultivation in South India.

## References

- Ajithkumar, B. 2005. Response of cauliflower (*Brassica oleracea* var *botrytis*) to weather with varying irrigation schedules and testing of vegetable model for middle Gujarat Agro-climatic Zone. Phd thesis, Anand Agricultural University, 235p.
- Callens, D., Rooster, L. and Reycke, L. 2000. Cultivar trial summer cultivation of cauliflowers: Fremont remains interesting, except for vein disease. *Proeftuinnieuws* 10(2): 16-17.
- Din, M., Qasim, M., Jan, N. E. and Faridullah, H. 2007. Response of different sowing dates on the Growth and yield of cauliflower. *Sarhad J. Agric.* 23 (2): 289-291.
- KAU [Kerala Agricultural University]. 2011. Package of Practices Recommendations: *Crops*. 14th Edition. Kerala Agricultural University, Thrissur, 360p.
- Kaur, H., Khurana, D. S. and Singh, K. 2007. Growth and yield of cauliflower (*Brassica oleracea* var. *botrytis*) as influenced by various planting dates in different intercrops. *Haryana J. Hortic. Sci.* 36 (3, 4): 324-325.
- Narayanankutty, C., Sreelatha, U., Gopalakrishnan, T. R. and Peter, K. V. 2012. Cabbage and cauliflower production in warm humid tropics of kerala. In: *Proceedings of 5<sup>th</sup> Indian Horticulture Congress*; 6-9 November 2012, Ludhiana, India, p. 213.
- Niewhof, M. 1969. Cole crops-botany, cultivation and utilisation. World Crop Book. Leonard Hill, London, p.196.
- Pradeepkumar, T., Babu, S. D., Aipe, K. C. 2002. Adaptability of cauliflower genotypes in the high ranges of Kerala. *J. Trop. Agric.* 40: 45-47.
- Yadav, A., Sharma, P., Korla, B. N. 1995. Response of cauliflower (*Brassica oleracea* var. *botrytis* L.) cultivars to different dates of planting. *Crop Res.* 9(3): 413-418.

**Table 1. Effect of sowing dates, varieties and their interactions on Days to curd initiation, Curd size index and Net curd weight of cauliflower**

Varieties	Days to Curd Initiation					Curd Size Index (Cm <sup>2</sup> )					Net Curd Weight (g)				
	Oct 1 <sup>st</sup>	Oct 15 <sup>th</sup>	Nov 1 <sup>st</sup>	Nov 15 <sup>th</sup>	Mean	Oct 1 <sup>st</sup>	Oct 15 <sup>th</sup>	Nov 1 <sup>st</sup>	Nov 15 <sup>th</sup>	Mean	Oct 1 <sup>st</sup>	Oct 15 <sup>th</sup>	Nov 1 <sup>st</sup>	Nov 15 <sup>th</sup>	Mean
Pusa Meghna	46.140	46.83	42.97	46.31	45.56	97.59	88.13	132.91	83.35	99.73	403.50	237.00	426.43	232.67	324.90
Pusa Sharad	79.50	73.39	63.70	49.70	66.57	20.45	26.56	24.53	13.08	20.84	60.00	83.57	93.66	104.00	85.31
Pusa Paushja	52.94	66.20	58.10	45.61	55.71	109.34	32.10	108.91	15.16	58.02	352.77	130.33	354.67	65.33	225.78
Pusa Hybrid 2	43.34	78.97	64.44	47.05	58.44	190.93	6.07	12.24	19.97	36.82	468.93	54.00	51.67	84.50	164.78
Pusa Shukti	48.80	65.60	48.60	37.04	50.00	103.74	34.30	103.74	17.47	58.06	308.00	136.00	308.00	104.00	214.00
NS 60 N	43.56	45.81	44.47	43.84	44.42	154.60	120.05	178.21	121.43	142.68	442.07	323.67	629.33	421.00	454.02
Himshort	37.22	39.23	38.16	40.27	38.72	61.52	95.99	145.90	79.34	93.40	213.3	237.33	429.70	268.67	287.26
Himlatha	48.90	45.48	52.42	49.07	48.97	109.93	94.09	126.10	105.77	108.91	395.87	268.67	422.00	331.33	354.47
Himpriya- 60	49.44	60.85	59.20	48.20	54.42	122.50	54.86	141.10	94.58	100.42	397.87	187.33	413.00	374.00	343.05
Indam 2435	44.48	43.95	53.77	53.39	48.90	89.47	133.95	113.09	65.74	99.13	274.67	369.00	324.80	176.67	286.28
G 45	41.03	47.23	51.53	51.43	47.80	104.15	110.33	125.46	110.83	112.61	357.73	291.34	430.00	370.00	362.27
White Snow	50.29	52.72	60.39	53.82	54.31	117.25	68.29	134.23	71.44	95.72	364.07	235.00	457.00	258.67	328.68
Mean	48.80	55.52	53.15	47.14		102.18	65.04	104.42	59.45		336.57	212.77	361.69	232.57	
CD (5%)	D				0.968					4.323					15.212
	V				1.374					7.077					19.570
	DxV				2.747					14.153					39.140

# Influence of spectral composition of light on the frequency of embryoid induction in anther culture of rapeseed

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### Abstract

This research deals with the action of light of different spectral composition on the frequency of appearance of morphogenic structures and their type during cultivation of rapeseed anthers. Blue-green light ( $\lambda_{\max}$  460 nm) was found to be more effective for such characteristic as the number of embryoids per single anther in comparison with yellow (510 nm) and red (610 nm) light. Newly developed haploid morphogenic structures were presented with embryoids, development of callus was not observed.

**Keywords:** *Brassica napus*, anther culture, glass filter, wavelength, morphogenic structure, embryoid

### Introduction

To make the process of development of new cultivars faster it is essential to raise initial homogeneous material as quick as possible. The problem is even more exasperating for those crops where hybrids are more prevalent than varieties. Anther and microspore cultures are the key techniques to overcome this challenge, especially for Cruciferous crops, as they permit to reach full homozygosity in one generation [3]. In spite that many aspects of anther *in vitro* response have already been investigated some issues are still understudied. One of them is influence of light of different wavelength on the morphogenesis *in vitro*. At the same time it is well known differentiated impact of rays of different wavelengths on that process *in vivo*. Although light is not usually considered to be necessary for the induction of androgenesis, for some species, like soybean [8] or *Citrus* [2], light of different quality was essential to produce morphogenic or embryogenic response.

The aim of our preliminary research was to study the action of rays of different spectral regions of visible light on the frequency of appearance of morphogenic structures and their type during cultivation of rapeseed anthers under artificial conditions.

### Material and Methods

Spring rapeseed (*Brassica napus* L.) accession D 28, received from Laboratory of Hybrid and Variety Breeding of Rapeseed of the Institute of Oilseed Crops, was used as the material. The inflorescences were cut before

the first flower flowering and kept for 2 days at low temperature of 5-8 °C in a refrigerator. After such pretreatment the buds of a proper size (with microspores primarily at the uninucleate stage) were selected, surface sterilized and anthers were picked out of the aseptic buds and planted onto an artificial nutrient medium in Petri dishes of 40 mm in diameter. The medium consisted of the basic MS salts with addition of extra calcium chloride, vitamins after Gamborg B5 medium, 800 mg/l glutamine, 100 mg/l serine, and 10 % of sucrose. Before autoclaving the medium was supplemented with 0.6 mg·l<sup>-1</sup> of benzyladenine and 0.2 mg·l<sup>-1</sup> of 2,4-dichlorophenoxyacetic acid. After anther planting the Petri dishes with anthers were kept in the special boxes covered with light filters BGF 22 (blue-green filter), YF 18 (yellow filter), RF 11 (red filter), capable to transmit light with wave length of  $\lambda_{\max}$  = 460, 510, and 610 nm respectively [9]. General illuminance of 5000 lx was supplied with fluorescent tube lamps. The cultivation temperature ranged from 23 to 27 °C. The day/ night cycle photoperiod was 18/6 h. During cultivation period (1 1/2 month) the number and type of newly developed structures were recorded and analyzed. The frequency of embryoids per single anther (as percent) was calculated as total number of emerged embryoids divided by the number of planted anthers and multiplied by 100. The evaluation of reliability of statistical differences between the treatments was performed according to Student t-test [7].

## Results and Discussion

As was shown at the end of the experiment new morphogenic structures were developed when anthers were cultivated behind the all glass filters used (Table 1). There were no differences observed in the frequency of responsible anthers. However rays of different wavelength exhibited different efficacy as to number of emerged embryoids per single anther. To be more precise, the least number of embryoids was developed in the treatment where anthers were kept behind a red filter, and the highest frequency was noted when cultivating anthers behind a blue-green filter.

As we can see from the table the highest frequency of embryoid emergence per single anther planted (about 40%) was observed when anthers were cultivated behind the glass filter with  $\lambda_{\max}$  of 460 nm (BGF 22). That was two times more if compared with filter of  $\lambda_{\max}$  460 nm and six times more if anthers were maintained behind the red filter of  $\lambda_{\max}$  610 nm. It means that the efficiency of spectral light for morphogenic structure induction (in terms of number of embryoids per anther) is inversely proportional to wavelength, being highest in the case of rays with shortest wavelength.

During cultivation of anthers in this experiment the structures of sporophytic origin were almost never observed. Only in the treatment with RF 11 glass filter such formations were sporadically noted. New morphogenic structures were usually presented with embryoids, while development of callus structures was not happening. It was obviously due to the phytohormonal and mineral composition of the used artificial medium, and not because of the influence of various spectral lights.

It is known that light of different spectral composition modifies hormone balance of plants [4]. In this way light is one of most important factors to alter plant growth pathway. As plants *in vitro* usually demand less light intensity than direct sunlight, it should be easier to change morphogenesis processes *in vitro* with light of different spectra. In our experiment blue-green light resulted in the highest frequency of embryoids per single anther. That corresponds with research of Konstantinova et al. [6] who demonstrated high efficiency of blue light which stimulated formation of vegetative buds in tobacco culture *in vitro* and with research of Cybularz-Urban et al. [1] who noted that blue treatments were effective in triggering *in vitro* photomorphogenesis of *Cattleya*. Even earlier Kataeva N. and Avetisov V. [5] showed that blue light was characterized as the main component of *in vitro* morphogenesis. Red light, however, has more controversial effect. We found that red light was less effective in anther culture of rapeseed while in the paper of

Cybularz-Urban et al. [1] red treatment was as much effective as blue one. Yet it could be explained as it is known that red light can enhance or reduce growth depending on how far the effected tissue is located in respect to endogeneous source of auxin [4].

## References

1. Cybularz-Urban T., Hanus-Fajerska E., Swiderski A. (2007) Effect of Light Wavelength on In Vitro Organogenesis of a *Cattleya* Hybrid. *Acta Biologica Cracoviensia, Series Botanica*. **49/1**: 113-118.
2. Germana M.A., Chiancone B., Iacona C., Muleo R. (2005) The effect of light quality on anther culther of *Citrus clementina* Hort. ex Tan. *Acta Physiologiae Plantarum*. **27** (4B): 717-721.
3. Haploids in Crop Improvement II (Biotechnology in Agriculture and Forestry). (2005). C. E. Palmer, W. A. Keller, and K. J. Kasha (Eds.). Springer. **56**. pp. 318.
4. Hormonal regulation of development II. The functions of hormones from the level of the cell to the whole plant (1984). T.K. Scott (Ed.). Springer Verlag. pp. 309.
5. Kataeva N.V., Avetisov V.A. (1981) Clonal propagation in tissue culture. (In Russian) *Kul'tura kletok rastenij*. Moscow, Nauka: 137-149.
6. Konstantinova T.N., Aksenova N.P., Sergeeva L.I., Chajlahjan M.H. (1998) Mutual influence of light and hormones in the regulation of morphogenetic processes in vitro culture. (In Russian) *Fiziologija rastenij*. **34** (4): 795-802.
7. Lakin G.F. (1980) Biometrics. (In Russian) Moscow. pp. 294.
8. Rodrigus L.R., Forte B.C., Oliveira J.M.S., Mariath J.E.A., Bodanese-Zanettini M.H. (2004) Effects of light conditions and 2,4-D concentration in soybean anther culture. *Plant Growth Regulation*. **44**: 125-131.
9. Veinberg I. (1967) The catalogue of color glass. (In Russian) Moscow, Mashinostroenie. pp. 62.

**Table 1. Influence of spectral composition of light on the formation of morphogenic structures in anther culture of rapeseed**

Treatment	Anthers planted, ea.	Frequency of embryoids per single anther, %	
		Mean	SEM
BGF 22 ( $\lambda_{max}$ 460 nm)	123	40,65	4,43
YF 18 ( $\lambda_{max}$ 510 nm)	102	20,59	4,00
RF 11 ( $\lambda_{max}$ 610 nm)	140	6,43	2,07

# Induction of androgenic callus in tropical early cauliflower (*B. oleracea var botrytis* L.)

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### Abstract

Haploid development technique in cauliflower has immense potentiality to accelerate hybrid breeding programme. The technology is being standardized on several Brassica species including cauliflower. However, this technology has not been undertaken in tropical Indian cauliflower before. Present study is conducted to understand the response of tropical cauliflower variety in anther culture. Two cauliflower varieties *i.e.* Sabour Agrim, a tropical Indian cauliflower variety and Pusa Hybrid 2, an snowball type cauliflower variety planted in the Vegetable Research Farm, Bihar Agricultural University, Sabour, Bhagalpur in the year, 2014-15. Anthers were collected at uni-nucleate stage of pollen grain. Culture mediums were prepared using MS salt and Gamborg B5 salt. Percent response for callus induction from anthers was varied between the genotypes. Highest response (31.97%) was observed for the variety Sabour Agrim. Among eight types of culture media composition only five were responded for callus induction. M8 medium (B5 salt + 100g/l sucrose + 1mg/l 2,4-D + 1mg/l NAA + 1mg/l BAP) was found highest responses for development of androgenic callus in tropical cauliflower.

### Introduction

Double haploid lines in Brassica species have been used for genetic mapping, linkage analysis and hybrid development. The technology is becoming popular because of its potentiality for rapid isolation of homozygous lines which reduces manpower, time and cost. Haploid production technology has been standardized in most of the *Brassica* vegetables like in cabbage (Cristea, 2013; Yang *et al.*, 2014) Cauliflower (Gu *et al.*, 2014); Broccoli (Yuan *et al.*, 2011) Brussel sprouts (Biddignton *et al.*, 1988) etc. Among all the brassica species *Brassica oleracea var botrytis* is most recalcitrant species and that is why a standardized techniques of other species or group within the species cannot be utilized for another species or group (Gu *et al.*, 2014). Double haploid production is a suitable alternative of traditional breeding. Genotypic effect and lower percentage of response for anther culture limits the practical implication of this technique in breeding programme. Identification of suitable genotypes well responsive for anther culture is a viable option.

During 1822, first time cauliflower was introduced in India by East India Company. Indian tropical cauliflower

was evolved by human selection over the period of time. Sawrup and Chatterjee (1972) classified Indian tropical cauliflower based on their maturity period and named as early cauliflower that matures in the month of September-October (curd initiates at around 25°C temperature). A diverse landraces has evolved in India over the year in tropical early cauliflower. Double haploid production in this group of cauliflower has high potentiality to un-reveal genetic information and accelerating hybrid breeding programme. The present experiment was laid out to understand the genotypic responses for anther culture and to identify suitable culture medium.

### Materials and methods

Two diverse genotypes of cauliflower namely Sabour Agrim, a tropical cauliflower variety developed from the locally adopted landraces of Bihar, India and Pusa hybrid 2, a snowball type cauliflower hybrid were planted in the Vegetable Research Farm, Bihar Agricultural University, Sabour in the year 2014-15. Laboratory experiment was laid out in factorial CRD with three replications. Eight media composition were taken into considerations to check the callusing out of anther (Table 1). Culture medium was prepared using MS salt with vitamins and Gamborg B5 Salt with vitamins along with different combination of growth regulators. Suitable anthers were collected at uninucleate stage of pollen grain then kept for 2 hrs under tap water than treated with 70% ethanol for 10 minutes followed by sodium hypochloride (0.4%) for 6 minutes and at last with mercuric chloride (0.1%) for 1 minute. Anthers were removed from the flower buds and then placed into culture medium. Pretreatment of anthers after inoculation was given at 35 ±1°C temperature for 24 hrs (Archer, 2002; Supena *et al.*, 2008). Immediate after pretreatment the culture was maintained in dark at 25±1°C temperature. After the emergence of the callus from the anther wall it was scored in percentage. Data analysis was carried out following Gomez and Gomez (1984).

### Result and Discussion

Significant variation was observed among the treatment for induction of callus from anthers (Table 2). Out of eight different types culture medium used in the present study five medium responded for callusing among which four were based on B5 salt and one was MS salt (Picture 1). Application of 5% coconut water had positive effect for the induction of callus. In respect to medium M3 and M6, medium M4 and M7 respectively were responded for callus induction (Table 1). Genotypic effect in response to application of coconut water was different. The efficiency of M3 medium (MS salt + 0.13mg/lit NAA + 0.4mg/lit 2,4-D + 5% coconut water) for variety Sabour Agrim 18.87%; while in M7 medium (B5 salt + 0.13mg/lit NAA + 0.4mg/lit 2,4-D + 5% coconut water) for variety Pusa Hybrid 2 was 23.75%.

Most of the treatments were responded one month after the inoculation of anthers. No significant variations were observed for number of days for 50% callusing. The size of the flower buds varied between the genotypes at uninucleate stage of pollen grain. 3-4mm size of bud was taken for variety Sabour Agrim and 5-6mm bud was for variety Pusa hybrid 2. Previous report found that size of the flower bud is an important factor for anther culture (Archar, 2002; Gu *et al.*, 2014; Keller and Armstrong, 1983). As cross pollinated in nature, the open pollinated variety Sabour Agrim showed plant to plant variation to some extent for size of flower bud.

Response of anther culture is governed by many factors. Genotype, temperature, pH, and time period even the anther density was found a key player for the induction of androgenic callus (Keller and Armstron, 1977). Response of genotype, culture medium and their interaction were found significant for percent callus induction (Table 3). Similar findings were also reported in cabbage (Magdi *et al.*, 2014). In the present experiment percentage of callus induced were varied from genotypes to genotype. Highest response (31.97%) was recorded for the variety Sabour Agrim (Fig. 1) in culture medium M8 (B5 salt + 100 mg/lit sucrose + 1mg/lit 2,4-D + 1mg/lit NAA + 1mg/lit BAP). Among all the responded medium M8 was found superior for callus induction in

both the varieties. Few culture mediums showed specificity to genotypes for callus induction. Mediums *i.e.* M3 and M4 only responded for variety Sabour Agrim. Though the anther sprouting was observed for variety Pusa Hybrid 2 in medium M3 (23.33%) and M4 (19.83%) but they did not turn up for callus production. Medium M7 only produced callus for Pusa hybrid 2.

## Conclusion

The effect of genotypes, culture medium and their interaction was found significant for percent response of culture medium and percent response of explants for induction of androgenic callus. The present result showed that M8 medium (B5 salt + 100 mg/l sucrose + 1mg/l 2,4-D + 1mg/l NAA + 1mg/l BAP) was found highest responses for development of androgenic callus in tropical cauliflower. Coconut water had positive effect for induction of callus from anthers.

## References

- Achar P.N. (2002). A study of factors affecting embryo yields from anther culture of cabbage. *Plant Cell Tissue and Organ Culture*. 69:183-188.
- Biddington, N.L., Sutherland, R.A., Robinson, H.T., 1988. Silver nitrate increases embryo production in anther culture of Brussels sprouts. *Ann. Botany*, 62, 181-185.
- Cogan, N.; Harvey, E.; Robinson, H.; Lynn, J.; Pink, D.; Newbury, H.J. and Puddephat, I (2001). The effect of anther culture and plant genetic background on *Agrobacterium* *ribozymes*-mediated transformation of commercial cultivars and derived double haploids in *Brassica oleracea*. *Plant Cell Reports*. 20:755-762.
- Cristea, T.O. (2013). The influence of pH on Microspore embryogenesis of White Cabbage (*Brassica oleracea* L.) *Not Sci Biol*, 5(4):485-489.
- Farnham, M.W. (1998). Double haploid broccoli production using anther culture: Effect of anther source and seed set characteristics of derived lines. *J.Amer. Soc. Hort. Sci.* 123(1):73-77.
- Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agricultural Research*. 2nd Edn., John Wiley and Sons Inc., New York, USA., ISBN: 13-9780471879312, pp: 13-175.
- Gu, H.; Zhao Z.; Sheng, X.; Yu, H.; and Wang, J. (2014). Efficient doubled haploid production in microspore culture of loose-curd cauliflower (*Brassica oleracea* var. *botrytis*). *Euphytica*. 195(3):467-75.
- Keller WA & Armstrong KC (1977) Embryogenesis and plant re- generation in *Brassica napus* anther cultures. *Can. J. Bot.* 55: 1383–1388.
- Keller, W.A. and Armstrong, C.K. (1983). Production of haploids via anther culture in *Brassica oleracea* var. *Italica*. *Euphytica*. 32(1):151-59.
- Keller, W.A., Armstrong, K.C., 1981. Production of anther-derived dihaploid plants in autotetraploid marrowstemkale (*Brassica oleracea* var. *acephala*). *Can. J. Genet. Cytol.* 23: 259-265.
- Magdi A.A. Mousa, Ashraf G. Haridy, Hassan S. Abbas and Mohammed F. Mohammed, 2014. Improved Androgenesis of Broccoli (*Brassica oleracea* var *italica*) Anthers Using Sucrose and Growth Regulators. *Asian Journal of Crop Science*, 6: 133-141.
- Prem, D; Gupta, K and Agnihotri, A (2005) Effect of various exogenous and endogenous factors on microspore embryogenesis in Indian mustard (*Brassica juncea* (L.) Czern and Coss), *In Vitro Cellular & Developmental Biology - Plant*, 41 (3): 266-273.
- Yuan, S.X.; Liu, Y.M.; Fang, Z.Y.; Yang, L.M.; Zhuang, M.; Zhang, Y.Y. and Sun, P.T. (2011) Effect of combined cold pretreatment and heat shock on microspore cultures in broccoli. *Plant Breed* 130:80–85.
- Supena EDJ, Winarto B, Riksen T, Dubas E, Van Lammeren A, Offringa R, Boutilier K, Custers J (2008) Regeneration of zygotic-like microspore-derived embryos suggests an important role for the suspensor in early embryo patterning. *J Exp*

**Table 1. Media composition used for anther culture in the experiment**

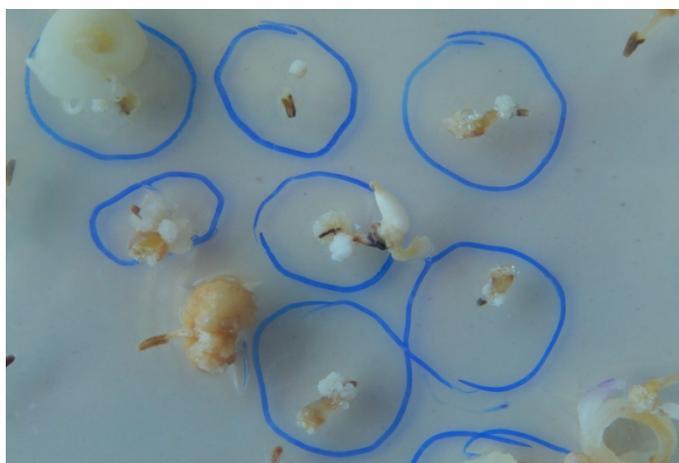
Sl. No.	Medium	Composition
1	M1	MS salt+100g sucrose/lit + 0.1mg/lit NAA+ 0.3mg/lit 24-D
2	M2	MS salt+100g sucrose/lit + 0.13mg/lit NAA + 0.4mg/lit 2,4-D
3	M3	MS salt+100g sucrose/lit + 0.13mg/lit NAA + 0.4mg/lit 2,4-D + 5% coconut water
4	M4	MS salt+200g/lit sucrose+0.02mg/lit TDZ+1mg/litIAA
5	M5	B5 salt + 100g/lit sucrose + 0.3mg/2,4-D +0.1mg/lit NAA
6	M6	B5 salt + 100g/lit sucrose + 0.3mg/lit NAA + 1mg/lit 2,4-D
7	M7	B5 salt + 100g/lit sucrose + 0.3mg/lit NAA + 1mg/lit 2,4-D + 5% coconut water
8	M8	B5 salt + 100g/lit sucrose + 1mg/lit 2,4-D + 1mg/lit NAA + 1mg/lit BAP

**Table 2. Response of culture medium and variety for callus induction**

Culture Medium responded	Percent of culture plate responded (%)		Percent of explants responded (%)	
	Sabour Agrim	Pusa Hybrid 2	Sabour Agrim	Pusa Hybrid 2
M3	23.33	23.33	18.87	0
M4	29.16	19.83	25.28	0
M5	24.72	36.66	17.55	19.24
M7	0	54.69	0	23.75
M8	44.48	46.63	31.97	29.08
SEd±	2.59**		1.51**	

**Table 3. Analysis of variance for Percent of cultured plate responded and percent of explants responded**

Percent of cultured plate responded				
Source of Variation	df	MST	F-value	SEm (±)
Medium	4	291.32	7.66**	4.594476
Genotype	1	757.0747	19.92**	1.83779
Medium X Genotype	4	700.2971	18.43**	9.188952
Error	20	37.99658		
Percent of explants responded				
Medium	4	464.7547	36.232**	2.669913
Genotype	1	230.5993	17.977**	1.067965
Medium X Genotype	4	851.1096	66.351**	5.339826
Error	20	12.82731		



**Fig: 1 Response of anthers of tropical cauliflower variety Sabour Agrim**

# Root tubers in plants of *Lunaria* L. genus and their inheritance

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### Abstract

Root system of two wild species of *Lunaria* L. genus was compared. It was found that *L. rediviva* (perennial species) had root tubers compared to *L. annua* (annual species). It was investigated the anatomical structure of this root formation and shown that it was typical for some cruciferous crops. The inheritance “root tubers” trait was studied. The dominance and monogeneity of this trait was revealed. Analysis of F<sub>2</sub> hybrids of *L. rediviva* × *L. annua* cross combination showed that “root tubers” and “plant development type” traits were independently inherited .

**Keywords:** *Lunaria*, root tuber, plant development type, inheritance

### Introduction

Honesty (*Lunaria* L.) is a new valuable cruciferous crop. It is known as ornamental garden plant with lilac, pink, red and white flowers. Light-green, green and dark green color of leaves creates a very good-looking plant. Due to biological peculiarities it can grow in shade and semi-shade. Honesty produces characteristic silver, translucent seed silicula that gave common name of this plant in England – money plant (penny plant) (Coombies, 2012).

However this crop is not only ornamental, but oilseed plant as well. *Lunaria*'s oil has valuable fatty acid content. It includes such important and rare acids as nervonic and erucic. Nervonic acid is mono-unsaturated Omega-9 fatty acid. It was found in sphingolipids of white matter human brain. It is also known that this acid takes part in biosynthesis of nerve cell myelin, constituent of membrane phospholipids (Sargent et al., 1994). The content of this acid in honesty oil may reach 20-25% (Cook et al., 1998). Honesty plants can be used as a source of 3-ketoacylCoA synthase (KSC) gene as well. This gene increases the content of nervonic acid in transformed yeast and transgenic plants (Guo Y et al., 2009).

Despite the diverse interest in this crop, information about it is scarce. The aim of this work was to study the anatomical structure of root tubers in *L. rediviva* plants and inheritance pattern for this trait in the interspecific crosses with *L. annua*.

## Material and Methods

Two wild species, which are included in *Lunaria* L. genus, were used as initial material. One of these - *L. rediviva* - is characterized by perennial development type. The second species - *L. annua* - is annual plant. These two species were crossed with each other. The F<sub>1</sub> plants of *L. rediviva* x *L. annua* cross combination were self-pollinated and individually harvested. Resulted seeds were sown in boxes under controlled indoor conditions. After formation of 3-4 well-developed pairs of true leaves, the F<sub>2</sub> plants were visually examined for the presence of tubers on the roots. Simultaneously, the type of plant development was recorded, taking into account that by this time the annual plants of F<sub>2</sub> population started to bloom. Anatomy of root tubers was analyzed using light-microscope technique. The  $\chi^2$  test was used for comparison of the segregation observed with the theoretically expected ratio (Griffiths et al., 2004).

## Results and Discussion

*L. rediviva*, being a perennial species, develops tubers on the roots in the first year after sowing the seeds (fig. 1). Characteristically, that such plant may have thickenings not only on the main root, but on the lateral roots as well (fig 2). It has been found that the anatomical structure of the root tuber in *L. rediviva* plant was typical for cruciferous crops, like radish, for example (Moore et al., 1995). In this case, storage parenchyma is localized in the xylem of the root, which is clearly seen from the Fig. 4.

F<sub>2</sub> hybrids of *L. rediviva* x *L. annua* cross combination were analyzed to establish the character of inheritance of the "root tubers" trait. The data are presented in the Table 1. As one can see from this table, the F<sub>1</sub> plants had root tubers, suggesting that the presence of tubers dominates over their absence. The majority of F<sub>2</sub> plants had root tubers, and about a quarter of them did not have such root formations. The  $\chi^2$  test statistically confirmed monogenic and dominant nature of the "root tubers" trait.

The data concerned to the joint inheritance of the "root tubers" and "plant development type" are presented in Table 2. Four phenotypes were found in F<sub>2</sub> population. Among F<sub>2</sub> plants, besides the parental phenotypes (perennials with root tubers and annuals without root tubers), perennials without root tubers and annuals with tubers were observed. Analysis of the F<sub>2</sub> population revealed dihybrid segregation concordant to the 9 : 3 : 3 : 1 ratio.

It should be noted that the intermediate phenotypes, that were found in our experiment, were not previously identified in natural environments. Because of artificial conditions and the early stage of F<sub>2</sub> plants identification, we cannot be sure that these intermediate phenotypes will confirm these characteristics at the end of the first year of life. It is possible that perennials without root tubers can develop them later but annuals with tubers will use nutrients of the root at the stage of flowering and pod formation.

So, we can draw some conclusions. Anatomical structure of the root tubers in *Lunaria* plants is typical for the cruciferous crops. Under artificial conditions *Lunaria* plants start to develop the root tubers at the stage of 4 true pairs of leaves. The presence of root tubers dominates over its absence and is inherited as a monogenic trait. "Root tubers" and "plant development type" traits have independent inheritance at early stage of plant development. Among F<sub>2</sub> hybrids of *L. rediviva* x *L. annua* cross combination two types of plants (annual plants with root tubers and perennial plants without root tubers) were found which are unknown in wild environments.

## References

- Coombes Allen J. e A to Z of plant names. – USA: Timber Press, 2012. – p. 312. – ISBN978–1-60469–196–2.
- Sargent J. R., Coupland K., Wilson R. Nervonic acid and demyelinating disease.//Med. Hypoth., 1994. – 42: 237–242.
- Cook C., Barne J., Coupland K., Sargent J. Effects of Feeding Lunaria Oil Rich in Nervonic and Erucic Acids on the Fa y Acid Compositions of Sphingomyelins from Erythrocytes, Liver, and Brain of the Quaking Mouse Mutant. Lipids. 1998. – 33: 993–1000.

Guo Y., Mietkiewska E., Francis T. et al. Increase in nervonic acid content in transformed yeast and transgenic plants by introduction of a *Lunariaannua* L. 3-ketoacylCoA synthase (KSC) gene. *Plant Mol. Biol.*, 2009. – 69: 565–575.

Griffiths A.J.F., Wessler S.R., Lewontin R.C., Gelbart W.M. and Suzuki D.T. 2004. *An introduction to genetic analysis*. 8th ed. WH Freeman and Company, New York: 800.

R. Moore, W. Dennis Clark, Kingsley R. Stern, Darrell Vodopich *Botany*, 1995. - USA: Wm. C. Brown Publishers. - 825 p.

**Table 1. F<sub>2</sub> segregation for «root tubers» trait in *L.rediviva* x *L. annua* cross combination**

F <sub>1</sub> phenotype	No. of F <sub>2</sub> plants	F <sub>2</sub> phenotypes	Segregation model	χ <sup>2</sup>
root tubers	134	108 roottubers 26 noroottubers	3 : 1	2,24

χ<sup>2</sup><sub>05</sub> (d.f.1) = 3.84

**Table 2. Inheritance of «root tubers» and «plant development type» traits in *L.rediviva* x *L. annua* cross combination**

F <sub>1</sub> phenotype	No. of F <sub>2</sub> plants	F <sub>2</sub> phenotypes				χ <sup>2</sup>
		withroottubers		withoutroottubers		
		perennial	annual	perennial	annual	
Observed segregation						
root tubers, perennial	134	87	21	15	11	7,36
Expected segregation						
root tubers, perennial	134	72	24	24	8	
Segregation model						
		9	3	3	1	



Figure 1. Root without tubers in *Lunaria annua* plant



Figure 2. Root with tubers in *Lunaria rediviva* plant

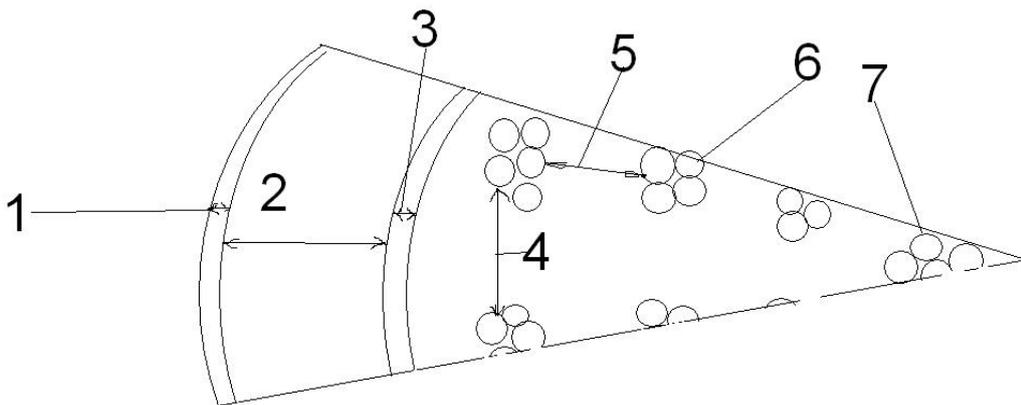


Figure 3. Anatomical structure of nodal root in *Lunaria rediviva*:

1 - epidermis; 2 –secondary phloem; 3 - cambium; 4 – distance between xylem rays; 5 –distance between groups of vessel elements; 6 –vessel elements; 7 –central cylinder



Figure 4. Main and lateral roots (with tubers) in *Lunaria rediviva* plant

# Induced with ethyl methanesulfonate the genetic variability of morphological traits in spring false flax (*Camelina sativa* L.)

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### Abstract

The spectrum and frequency of morphological mutations after seed treatment with ethyl methanesulfonate at 0,01-0,5% concentrations was studied in spring false flax. 9 types of heritable changes in M<sub>2</sub> generation were revealed. The maximum frequency of mutations was observed at 0.5% concentration of mutagen but valuable for breeding practice traits were found at less concentrations of ethyl methanesulfonate. The mutations of modified leaf contour and large seeds were used for development of new false flax varieties.

**Keywords:** *Camelina sativa*, ethyl methanesulfonate, M<sub>2</sub> generation, morphological mutation, frequency and spectrum

### Introduction

Now, the needs of vegetable oil are based on growing a small amount of oilseeds. However, the development of food and other industries requires diversifying the range of oils. One of the solutions of this problem is unique in the fatty acid ratio spring false flax oil. Its seeds contain 40-46% oil, which finds many uses. False flax oil is used as a technology in the steel industry; for making varnish; in perfumery and cosmetics industry, including soap; in food (especially as a dietary product), begins to be used in the medical industry with curative purposes; as a component in massage creams; in cosmetics and aromatherapy (Gugel and Falk, 2006).

Spring false flax has several positive biological features. The first is a short growing season. In the most growing areas it matures within 60-90 days. By its biological nature, false flax is less demanding to growth conditions than other oilseeds. It has high cold resistance, and, at the same time, is drought-resistant plant. In addition, unlike other cabbage family crops, false flax at all stages of development is not colonized by pests and not damaged by diseases that does not require the use of pesticides (Francis and Warwick, 2009).

Thanks to low-cost cultivation and thus little impact on the environment, false flax is becoming a popular crop for the production of organic products. A comprehensive applicability of false flax oil in food, cosmetic, perfume

industry, in medicine and as a feedstock for biodiesel production has a huge market potential and making false flax a crop of the future (Hixson et al., 2014).

Chemical mutagenesis method allows in a short time to create a new source material characterizing by diverse morphological, physiological and biochemical characteristics. The results of studies on the effectiveness of inducing mutations in different genotypes of spring false flax after seed treatment with ethyl methanesulfonate (EMC) are presented in this paper.

### Material and Methods

Spring false flax mature seeds of Mirazh and Stepovyi 1 varieties and K-4154 sample were treated with 0,01, 0,05, 0,1 and 0,5 % of ethyl methanesulfonate for 18 hours. 200 seeds were used for every treatment. After treatment the seeds were washed to remove the mutagen. Seeds of every  $M_1$  plant were sown separately in individual plant-to-progeny rows. In the  $M_2$  generation the majority of visually selected mutants was selfed and advanced from  $M_2$  to  $M_3$ . Every mutant line was raised along with its parent. Mutation frequency was calculated as a percent of families with mutant plants selected in  $M_2$  to the total number of analyzed families.

### Results and Discussion

Morphological mutations were submitted to 3 groups and 9 types of genetic changes: mutations of chlorophyll deficiency (4 types), mutations of vegetative organs (3) and mutations of generative structures (2) (Table).

Chlorophyll mutations were characterized by complete or partial violation of the synthesis of chlorophyll in plants. It is known that they lead to death or reducing the viability of plants, therefore they are not interesting for breeding practice. But, due to the high frequency of their emergence and ease of account, chlorophyll mutations can be used as tests of mutagenic action efficiency and are often in direct correlation with hereditary variability of morphological and physiological characteristics. Using this test, we can determine the optimum dose and other conditions of the mutagen action.

Chlorophyll mutations, identified in  $M_2$  of spring false flax, differed in appearance and degree of plant depression. Mutation of *albina* type was characterized by white or whitish-yellow cotyledons (Fig. 1). The mutation was always lethal. Even if the plant started to develop the first pair of true leaves of green color, it did not survive later. *Xantha* plants had yellow or yellowish cotyledons and true leaves. These plants were inhibited in growth, ripened earlier, were low-yield and sometimes died. *Virescent* mutation was characterized by the fact that chlorophyll appearance of *xantha* type disappeared, but the plants retarded in development. The plants had lighter color of leaves but were productive. The plants of *viridis* type had light-green, greenish-yellow or pale green leaves and almost did not reduce productivity.

Mutations with violation of chlorophyll synthesis were found for all studied genotypes of spring false flax. As is shown in the Table, the spectrum of mutations differed slightly, but was wide enough. The maximum frequency of such mutations was observed at concentrations of 0,5% mutagen treatment. In this case the percentage of chlorophyll disorders that significantly affect the development of plant, inhibiting it, or even lethal, is greatly increased. Thus, at the maximum concentration of mutagen for Stepovyi 1 variety the mutation of *albina* type with the frequency of 50 % was revealed, and for Mirage variety with frequency of 10,0% – the mutation of *virescent* type. *Xantha* mutation was found for all samples with a maximum rate from 5,88% for Mirage to 25% for the sample K-4153. The mutation of *viridis* type was derived in all samples. The frequency of this mutation

was increased with increasing concentration of mutagen and reached 20% for Mirage, 25,0% for K-4153 and 50,0% for Stepovyi 1.

Mutations of vegetative organs included three types of changes. Mutation of leaf contour has led to changes in shape of leaf margin. It was a rare and was revealed only in two families of K-4153 sample (Fig. 2). Mutation associated with the lack of pubescence was unique for Stepovyi 1 variety. It was characterized by glabra surface of leaves and stems in comparison with densely pubescent leaves in the initial genotype. Dwarf mutants were found with high frequency in all studied genotypes (Fig. 3). As chlorophyll mutations, their frequency was increased with increasing the concentration of ethyl methanesulfonate.

Mutations of generative structures included the heritable changes of flowers and seeds. In the first case the modification of flower structure, which had accrete petals, was revealed. This mutation was found in two families of K-4153 at a concentration of 0,1% mutagen. Mutation of seed size was found in six families of Mirage variety at a concentration of 0,1% ethyl methanesulfonate. In this case, the mutants exceed per 0,4-0,6 g the original genotype by weight of 1,000 seeds.

The latest mutation was of particular interest to breeders. The mutant having large seeds, became the founder of the Prestige variety. Another mutation associated with the change of the leaf contour was used as a marker trait when Zeus variety was created. Both varieties are included in the Register of Plant Varieties of Ukraine (Komarova, 2010).

Summarizing the data, it is established that seed treatment of spring false flax seeds of three samples at 0,01-0,5% concentrations of ethyl methanesulfonate led in  $M_2$  to 9 types of changes in morphological characters. The largest group of hereditary changes was chlorophyll mutations. It is proved that the mutation rate greatly depends on the genotype and concentration of mutagen. Mirazh variety was the most mutable. The overall frequency of mutant families for this genotype was about 20%. The optimum concentrations of mutagen to induce genetic changes that can be used as a marker or economically valuable traits were revealed.

## References

- Francis A., Warwick S.I. (2009). The biology of Canadian weeds. *Camelina alyssum* (Mill.) Thell.; *C. microcarpa* Andr. Ex DC.; *C. sativa* (L.) Crantz. Can. J. Plant Sci. **89**:791-810.
- Gugel R. K., Falk K.C. (2006). Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. Can. J. Plant Sci. **86**:1047-1058.
- Hixson S.M., Parrish C.C., Anderson D.M. (2014). Use of camelina oil to replace fish oil in feeds for farmed salmonids and Atlantic cod. Aquaculture. **431**:44-52.
- Komarova I.B. (2010). Trait variability of a spring false flax and creation of the source material by a method of chemical mutagenesis. Thesis for a Candidate of Agricultural Sciences degree. Grain Farming Institute of UAAS, Dnipropetrovsk. (in Ukrainian).



**Figure 1.** Mutation of *albina* type



**Figure 2.** Mutation of leaf contour



**Figure 3a.** Dwarf mutant (right) compared to the initial sample (left) of spring false flax



**Figure 3b.** Dwarf mutant of spring false flax in blooming

**Table 1. Mutation frequency of morphological traits induced with EMS in M<sub>2</sub> of spring false flax, %**

Type of mutation	Samples and EMS concentrations, %														
	Mirage					Stepovyi 1					K-4153				
	0	0,01	0,05	0,1	0,5	0	0,01	0,05	0,1	0,5	0	0,01	0,05	0,1	0,5
<b>Chlorophyll-deficient mutations</b>															
<i>Albina</i>	0	0	0	0	0	0	0	0	0	50,00	0	0	0	0	0
<i>Xantha</i>	0	3,57	1,22	5,88	5,00	0	0	0	3,77	0	0	2,44	5,95	0,98	25,00
<i>Virescent</i>	0	0	1,22	5,88	10,00	0	0	0	0	0	0	0	0	0	0
<i>Viridis</i>	0	1,79	3,66	5,88	20,00	0	0	0	0	50,00	0	1,22	0	0,98	25,00
Total	0	5,36	6,10	17,64	35,00	0	2,30	0	3,77	100,00	0	3,66	5,95	1,96	50,00
<b>Mutations of vegetative organs</b>															
Modification of leaf contour	0	0	0	0	0	0	0	0	0	0	0	1,22	0	0	0
Lack of pubescence	0	0	0	0	0	0	0	0	0	50,00	0	0	0	0	0
Dwarf	0	0	1,22	5,88	15,00	0	1,15	0	0	50,00	0	0	0	1,96	25,00
Total	0	0	1,22	5,88	15,00	0	1,15	0	0	100,00	0	1,22	0	1,96	25,00
<b>Mutations of generative structures</b>															
Accrete petals	0	0	0	0	0	0	0	0	0	0	0	0	0	0,98	0
Large seeds	0	0	0	5,88	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	5,88	0	0	0	0	0	0	0	0	0	0,98	0
Number of families	112	112	164	102	40	186	174	102	106	4	174	164	167	198	8
Total mutations, %	0	5,36	7,32	29,4	50,0	0	3,45	0	3,77	200,00	0	4,88	5,95	4,90	75,00
Sp	0	2,13	2,03	4,51	7,91	0	1,38	0	1,85	70,71	0	1,68	1,83	1,53	15,31

## GENERAL INFORMATION ON BRASSICA

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### Brassicaceae are simply beautiful!

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#### Introduction

It is estimated that a worldwide rather known family of Brassicaceae Burnett (syn. Cruciferae Juss.) comprises about 370 genera and between 4,000 and 4,100 species of mostly herbaceous annual, biennial and perennial plants, as well as a certain number of shrubs and trees, mostly in warmer climates (Angiosperm Phylogeny Group 2009). It includes numerous economically important crops, cultivated for food, feed and various non-food uses. Among the most renowned examples are rapeseed (*Brassica napus* L.), cabbage (*Brassica oleracea* L.), in a broader sense and with several conspecific taxa or cultivar groups, and mustards (*Sinapis* spp.). It is noteworthy to mention that a member of this family is thale cress (*Arabidopsis thaliana* (L.) Heynh.), one of the first and the most significant model plant species (Meyerowitz 2001).

There are many similarities between the families Brassicaceae and Fabaceae Lindl. Along with the facts that their most widely grown crops, in many cases, share the same centres of origin (Zohary et al. 2012), that their fruits and seeds have similar morphology or that both consists of wild, weedy, edible and industrially remarkable species, they share one more common characteristic, however obscured by all their uses and diversity: they all may be, in their own way, considered ornamental (Mikić 2015). Thus, in the form of this very brief overview, we desired to present only few of the countless brassica species that have or may have a decorative use.

#### Wild, but verily beauties and certainly not beasts

Purple rock cress (*Aubrieta deltoidea* (L.) DC.) (Fig. 1, top, left), also known as lilacbush and rainbow rock cress, owes its scientific name to Claude Aubriet (1651–1743), a French painter, famous for his miniatures of flora and fauna, seemingly not completely without reason (Fig. 2). This perennial plant is native to Southeast Europe and may be found from Italy to Iran. Because of its growing habit, namely a carpet-like groundcover of leaves and lavender-, lilac- and rose-coloured flowers, it is frequently used as an ornamental in some regions (Rollins 1993). One of them is the United Kingdom, where breeding purple rock cress is perhaps among the most advanced and with the cultivars such as 'Argenteovariegata' or 'Red Cascade' widely grown.



**Figure 1. Wild, weedy, cultivated... And all beautiful: (top, left) *Aubrieta deltoidea*, (top, right) *Brassica napus*, (bottom, left) *Brassica nigra* and (bottom, right) *Orychophragmus violaceus***



**Figure 2. A painting of (left) turnip rape (*Brassica rapa* L.) and (right) cabbage (*Brassica oleracea* L. var. *capitata* L.) by Claude Aubriet (1651-1743); by courtesy of AllPosters.com**

The original homeland of Chinese violet cress (*Orychophragmus violaceus* (L.) O. E. Schulz) (Fig. 1, bottom, right) is, as its English name suggests, East Asia or, more specifically, a belt from eastern China to Korea. Preferring open spaces and abundant sunlight, it is often found in the grasslands, on the hill slopes or by the roadsides (PFAF 2012). One of its main uses in China is as a vegetable, with leaves and flowers eaten as salad or cooked. In addition and in West Europe and USA, it also became popular as an ornamental, with some initial breeding efforts bringing forth the first commercial cultivars with a range of violet petals hue, such as 'February Orchid' (BES 2016).

**The noble and learned ladies and their rude but handsome companion**

Rapeseed (Fig. 1, top, right) is surely one of the best studied Brassicaceae species and with a longest tradition

of research, including whole-genome sequencing (Choi et al. 2007), crop history (Chalhoub et al. 2014), diverse molecular-level in-depth analyses (Szadkowski et al. 2010), nutritional quality (Nesi et al. 2008) or biofuel use. The same may be said for Indian (*Brassica juncea* (L.) Czern.), white (*Sinapis alba* L.) and other mustards (Lionneton et al. 2004, Nelson and Lydiate 2006). It is most likely that all these various economically significant applications that are responsible for the lack of primarily ornamental cultivars of either rapeseed or mustards, but the scenery of a vast field of these crops in bloom under a clear and blue sky, equally common for Saskatchewan in Canada, Ireland and East Anglia in UK, Burgundy in France, Serbia in the Balkan Peninsula, eastern Ukraine and southern Russia and numerous regions in China, makes them, makes them beauty as they are and lift both heart and spirit of many a scientist or farmer worldwide.

Black mustard (*Brassica nigra* L.) (Fig. 1, bottom, left) is a native to South Europe, but its domestication probably took place in the Indian Sub-continent. There, it is widely cultivated as a spice crop and used in traditional cuisine, propelling both genetic and breeding research (Negi et al. 2004). On the other hand, in its original homeland and many other temperate regions, black mustard is a notorious weed in cereal, legume and other crops. However, it was relatively recently that its possible phytoremediation role has been emphasized (Szczygłowska et al. 2011). In that way, many a tall and branched black mustard plant, that one sees growing along the roads and railways, in the waste-filled canals or on the devastated and overexploited soils, may be considered an environment-friendly golden coin in the midst of mud and dirt.

#### **Instead of conclusions: To eat or not to eat but merely sit, watch and adore?**

A specific morphology of two of the most significant vegetable fruits across the world, namely cabbage and kale (*Brassica oleracea* L. var. *sabellica* L.) offers a lot of opportunities for breeding and developing exclusively ornamental cultivars, especially in China, Japan, UK and USA (Gibson and Whipker 2001, Meng et al. 2005). By applying a thoroughly-designed crosses and targeting specific traits, it is possible to achieve various ideotypes (Fig. 3). Most of them have a large number of leaves arranged on a relatively small length and thus form very dense rosettes. Their leaf size and shape is very variable, while the leaf colour may range from purely white ('Osaka White', 'Pigeon White'), over pink ('Colour-up Pink'), lavender, purple and violet to blue and red ('Nagoya Red', 'Tokyo Red').



**Figure 3. Variability of ornamental cabbage and kale cultivars; by courtesy of (left) Amy Cicconi and (right) Harris® Seeds**

A specific agronomy is needed for growing such advanced cultivars in the gardens, by adjusting the sowing time, temperature and water supply, leading to the best decorative performance, in terms of phenotype and both length and intensity of rosette colour (Whipker et al. 1994, Whipker et al. 1998).

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## References

- Angiosperm Phylogeny Group (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161:105-121
- BES (2016) February Orchid *Orychophragmus violaceus*. Brown Envelope Seeds (BES), Turk Head, West Cork
- Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B, Corréa M (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Sci* 345:950-953
- Choi SR, Teakle GR, Plaha P, Kim JH, Allender CJ, Beynon E, Piao ZY, Soengas P, Han TH, King GJ, Barker GC (2007) The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. *Theor Appl Genet* 115:777-792
- Gibson JL, Whipker BE (2001) Ornamental cabbage and kale cultivar comparison study: Growth characteristics and response to daminozide and uniconazole foliar sprays. *HortTechnol* 11:376-380
- Lionneton E, Aubert G, Ochatt S, Merah O (2004) Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*). *Theor Appl Genet* 109:792-799
- Meng ZQ, Xu DS, Wang JZ (2005) Tissue culture of ornamental kale. *J Wuhan Univ Nat Sci Ed* S2
- Meyerowitz EM (2001) Prehistory and history of Arabidopsis research. *Plant Physiol* 125:15-19
- Mikić A (2015) All legumes are beautiful, but some legumes are more beautiful than others. *Legum Perspect* 8:26-27
- Negi MS, Sabharwal V, Bhat SR, Lakshmikumaran M (2004) Utility of AFLP markers for the assessment of genetic diversity within *Brassica nigra* germplasm. *Plant Breed* 123:13-16
- Nelson MN, Lydiate DJ (2006) New evidence from *Sinapis alba* L. for ancestral triplication in a crucifer genome. *Genome* 49:230-238
- Nesi N, Delourme R, Brégeon M, Falentin C, Renard M (2008) Genetic and molecular approaches to improve nutritional value of *Brassica napus* L. seed. *C R Biol* 331:763-771
- PFAF (2012) *Orychophragmus violaceus* (L.) O. E. Schulz. Plants For a Future (PFAF), Dawlish
- Rollins RC (1993) Brassicaceae. The Mustard Family. In: Hickman JC (ed) *The Jepson Manual: Higher Plants of California*. University of California, Berkeley
- Szadkowski E, Eber F, Huteau V, Lodé M, Huneau C, Belcram H, Coriton O, Manzanares-Dauleux MJ, Delourme R, King GJ, Chalhoub B, Jenczewski E, Chèvre A-M (2010) The first meiosis of resynthesized *Brassica napus*, a genome blender. *New Phytol* 186:102-112
- Szczygłowska M, Piekarska A, Konieczka P, Namieśnik J (2011) Use of brassica plants in the phytoremediation and biofumigation processes. *Int J Mol Sci* 12:7760-7771
- Whipker BE, Eddy RT, Hammer PA (1994) Chemical growth-retardant height control of ornamental kale. *HortSci* 29:329-329
- Whipker BE, Gibson JL, Cloyd RA, Campbell CR, Jones R (1998) Success with ornamental cabbage and kale. *Hortic Inf Leaflet* 507:1-9
- Zohary D, Hopf M, Weiss E (2012) *Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin*. Oxford University Press, Oxford

# CRUCIFERAE NEWSLETTER Nr. 36

## Instructions to the authors – 2016

**Deadline for contribution submission: April 1<sup>st</sup> 2017**

The next issue of the Cruciferae Newsletter (vol. 36) will be published online during Spring 2017 from the Brassica website (<http://www.brassica.info/info/publications/cruciferae-newsletter.php>). Online process will ensure rapid publication of your contribution. Therefore, we should be grateful if you would, please, follow the instructions below.

1- All contributions should be written in **English**.

2- Authors should submit manuscripts only by email to [cruciferaenewsletter@rennes.inra.fr](mailto:cruciferaenewsletter@rennes.inra.fr). A manuscript file in Microsoft Word (or some other word processing format) is required. The manuscript file must be named as following: Full name of the first author\_Year of submission.doc or .rtf.

3- As previously contributions must not exceed **2 pages**, including tables, figures and photographs. **Arial 10** character is expected with single spacing (**please use the submission form below**).

4- The heading of the paper must be written in boldface letters and must include the title (1<sup>st</sup> line), followed by the author names (lines below) and their address (3<sup>rd</sup> lines) with the email address of the corresponding author.

5- Tables, figures and photographs must be included in, or at the end of the text.

6- While submitting their contributions, authors should mention **one of the listed topics** that is the most relevant to their work (see the list below), in order to facilitate the editing process.

7- All papers are published on their author's responsibility.

### **List of selected topics (please, choose one topic for submission)**

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**Table 1. Title**

**Figure 1. Title**