



INFLUENCE OF PLANTING DENSITY OF MONOGERM AND MULTIGERM RED BEET STECKLINGS ON THE SEED STALKS CHARACTERISTICS

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Abstract

The aim of this research was to study the effect of stecklings planting density on the seed stalk architecture, cluster yield and seed quality of 20 mono – and multigerm breeding lines and cultivars of red beet. The study included the new monogerm lines – AR79 A and AR79 B – breed in the Unit of Genetics Plant Breeding and Seed Science at the University of Agriculture in Kraków. The experiment was conducted in the years 2010 and 2011, in the open field conditions. Two planting densities of stecklings: 50 × 25 and 50 × 40 cm, were applied. Standard crop management practices, as recommended for red beet seed production under Polish conditions, were followed. The increase in plants interspacing from 50 × 25 to 50 × 40 cm led to the lower height, but twice higher number of shoots per plant, lower number of single, but higher number of bush and bush with main stem seed stalks, twice higher yield per plant as well higher thousand-seed weight and germination capacity. A large variation between genotypes in the features of seed-bearing plants, such as morphological structure, clusters yield and seed quality, were noted. Compared to other genotypes, the new monogerm breeding lines AR79 A and AR79 B formed very high, mostly bush or bush with main stem seed plants developing one of the largest numbers of unluckily, late-ripening shoots, yielded on the average level and producing the seeds of relatively low quality. The research has expanded the knowledge about

the generative development of red beet. The results will facilitate Polish breeding programs aimed at the selection of monogerm red beet lines.

Key words: *Beta vulgaris* L., clusters, germination, seed production, breeding, seed yield

INTRODUCTION

Red beet (*Beta vulgaris* L.), belonging to the Chenopodiaceae family, is one of the major Polish vegetable crops in terms of production and consumption. Red beet roots are a component of traditional dishes in the Central and Eastern Europe. The vegetative growth of this vegetable has low requirements and its roots can be stored for many months. Red beet is a great source of beneficial for human health nutrients, such as microelements, vitamins and antioxidants, especially betalains, which labelled as E-162, are used as a natural dye for many food products (Neelwarne and Halagur 2013, Ravichandran *et al.* 2013, Stagnari *et al.* 2014).

Nowadays, the red beet producers, instead of the traditional open-pollinated cultivars, prefer hybrid cultivars, whose production is based on cytoplasmic male sterile breeding lines. Moreover, the precision seed sowing that allows avoiding the plant thinning is possible only in the case of the monogerm clusters of good quality. Therefore, the modern breeding programs consider the above trends aimed at the selection of new monogerm red beet lines for hybrids performance (Goldman and Navazio 2008).

In Poland, the red beet seed production is associated with a risk of low clusters yield and seed quality. The knowledge about the factors affecting the generative development of red beet is still quite poor. The environmental conditions of Poland, such as air temperature and precipitation during the growing period have a decisive influence on the cluster yield and seed quality of red beet (Wawrzyniak 1991a, 1991b). A strong effect of climatic and soil conditions on the yield and quality of sugar beet clusters was reported by Mikita and Gutmański (2002a, 2002b). However, the growth of beet seed-bearing plants also largely depends on the genotype as well the agronomical factors, such as planting density, steckling size, harvesting date or fertilization that modify the plant morphology, ripening uniformity and seed quality. Michalik and Kozak (1993) observed lower yielding and shorter seed plants of red beet breeding lines when compared to the hybrids. A higher seed quality of red beet cultivars than breeding lines was presented by Michalik (1993). Apostolides and Goulas (1998) found the differences in seed size among tested sugar beet cultivars. Several studies reveal the effect of planting density of beet stecklings on the seed plants growth and branching as well as the clusters yield and seed quality. Balan and Zagorodnii (1986) and He-

mayati *et al.* (2008) noted that the decrease in sugar beet plant density led to the increase in multi-branch plants causing the changes in morphological structure of seed-bearing plants. Nicolau (1978), Balan and Zagorodnii (1986), Podlaski (1987), Leibovich *et al.* (1993) and Hemayati *et al.* (2008) observed a significant effect of increase in sugar beet plants interspacing on the increase in cluster yield per plant. Also for the monogerm forage beet Bordei and Tapus (1981) noted the rise in yielding with the decrease in planting density. On the other hand, Scott and Longden (1973) and Kaw and Mir (1975) revealed the increase in clusters yields with the increasing in plant density. Studies presented by Slavov *et al.* (1997), Pospisil and Mustapic (1999) and Hemayati *et al.* (2008) suggest that with the decrease in sugar beet seed plants density, the cluster size also increase. Against, Lachowski and Howwicki (1973) reported that the increase in sugar beet seed plant density did not influence the thousand-seed weight. According to Slavov *et al.* (1997) and Hemayati *et al.* (2008) the clusters germination rate increases, as the plants interspacing increases. Contrariwise, Scott and Longden (1973) noted the improvement in germination vigour with the plant density increasing.

The aim of this experiment was to study the impact of planting density of red beet stecklings on the seed plants morphology as well as the yield of clusters and seed quality. 20 genotypes, including mono – and multigerm, breeding lines and cultivars, were tested. The stecklings were planted in the rows spaced 50 cm apart. The distances between stecklings were 25 or 40 cm. The investigation of generative development of red beet genotypes will be supportive for the breeding programs and seed production of this species.

MATERIAL AND METHODS

The experiment was performed in the years 2010 and 2011 at the Experimental Field of the Unit of Genetics, Plant Breeding and Seed Science at Prusy near Krakow, southern Poland (50°06'N, 19°96'W). The seed-bearing plants of red beet were cultivated on the black degraded soil. The nutrient content in the spring before planting was 60 mg N-NO₃ dm⁻³, 100 mg P dm⁻³, 120 mg K dm⁻³, 1150 mg Ca dm⁻³, 80 mg Mg dm⁻³ and pH 6.2 in the year 2010, and 65 mg N-NO₃ dm⁻³, 96 mg P dm⁻³, 125 mg K dm⁻³, 1200 mg Ca dm⁻³, 80 mg Mg dm⁻³ and pH 6.1 in the year 2011. The trial field was managed following standard crop practices as recommended for red beet seed production under Polish conditions. The experimental design was a split-plot system with three replications. In the study, two factors were evaluated; the first was the planting density of stecklings and the second was the genotype. The stecklings were planted at the beginning of April in the rows spaced 50 cm apart. The spaces between stecklings were 25 or 40 cm. In every replication, 5 stecklings spaced 25 cm apart and 5 stecklings spaced 40 cm apart, for each genotype, were planted. The plant material tested in

the experiment consisted of 20 genotypes of red beet (*Beta vulgaris* L.), including three monogerm cytoplasmic male sterile (CMS) breeding lines A: AR79 A, 279 mono A and W411 A, and their respective maintainer fertile lines B: AR79 B, 279 mono B and W411 B as well as three multigerm CMS lines A: 218 A, 357 A and 391 A and their respective maintainer fertile lines B: 218 B, 357 B and 391 B. Furthermore, eight cultivars, including five monogerm seed: Modana, Mone-ta, Monika, Monorubra and Patryk, and three multigerm seed: Astar F₁, Okragly Ciemnoczerwony and Polglob F₁.

The air temperature and the total rainfall recorded during the experiment are presented in the Table 1. Mean monthly air temperature during the 2010 growing period (from April to September) was comparable to the average from the years 2002-2011, but lower almost by 1°C than in the year 2011. In the year 2010, the rainfall totals during the growing period were 80% higher comparing to the years 2002-2011 and 85% higher than in the year 2011. In May, August and September 2010, the precipitation totals were 2.4-fold higher and in June almost twice higher, comparing to the average from the years 2002-2011.

Table 1. Mean monthly air temperature and total rainfall during the years 2010-2011 growing period

Months	Temperature (C°)			Rainfall (mm)		
	Year 2001	Year 2011	Mean for 2002-2011	Year 2010	Year 2011	Mean for 2002-2011
April	8.7	10.8	8.9	37.6	68.2	32.1
May	12.5	13.3	13.4	205.2	52.8	85.0
June	17.0	18.0	16.9	138.0	35.6	76.0
July	20.2	17.4	18.2	115.4	191.8	118.6
August	18.1	18.9	18.4	163.0	56.6	68.5
September	11.8	15.2	13.2	107.8	9.2	45.2
Mean temperature and rainfall totals for growing period	14.7	15.6	14.8	767.0	414.2	425.5

The ripened seed stalks were hand-collected between the middle of August and the end of September. The harvest started when the clusters at the base of each branch were brown and mature. The seed-bearing plants were air-dried hanging under the roof for a week; next the clusters were hand-threshed and air-dried for a month at a temperature of 20-25°C. The observations performed during the harvest included the height (cm) of the longest shoot of each plant and the number of shoots per plant. The seed stalk architecture was determined ac-

cording to Janas and Grzesik (2007); the percentage of the plant assigned to one of three morphological types: “single”, “bush” or “bush with main stem” was calculated. After drying, the yield of clusters per plant (g) was measured. Subsequently, the thousand-seed weight (g) was estimated based on the International Rules for Seed Testing (ISTA 2011). The germination test for the evaluation of germination capacity (GC) and mean germination time (MGT), according to the International Seed Testing Association recommendations (ISTA 2011), was performed after 6 months of the cluster storage. The GC as the percentage of normal seedlings after 14 days of germination was counted using the ISTA Handbook for Seedling Evaluation Guidelines (Don 2009). For the MGT calculation the seedlings that initiated germination with a protruded radicle of 2 mm long were counted daily, at the same time, from the moment of planting until the final count made 14 days after planting. The MGT was calculated using the following formula: $MGT = \Sigma (D \times N) / \Sigma N$; where N is the number of clusters which germinated on day D and D is the number of days counted from the beginning of germination.

Statistical analysis was performed using the software STATISTICA (version 12.0). The data collected during two years of two-factorial experiment were subjected to a general analysis of variance (ANOVA, $P < 0.01$). To estimate the significant differences between the means for the height of seed stalks, number of shoots, seed stalk type, yield of clusters, thousand-seed weight, MGT and GC, the Duncan’s test at $p = 0.05$, was applied.

RESULTS AND DISCUSSION

The red beet stecklings interspacing applied in the present research significantly affected the seed-bearing plants morphological structure (Table 2). The increase in planting density of stecklings from 50×40 to 50×25 cm led to the increase in seed stalks height from 112.8 to 116.6 cm. Also the year of experiment significantly affects the plant height. In the year 2010, when the rainfall totals during the growing period was nearly twice higher than in the year 2011, the plant were higher, compared to the year 2011. The studied genotypes also notably differed in the seed-bearing plants height. Compared to the lines, the cultivars formed the seed stalks with higher growth that varied between 115.8 and 143.7 cm. In five out of six tested breeding lines, the plant height ranged from 97.2 to 108.5 cm. The exception was the seed stalks of monogerm lines AR79 A and AR79 B, whose height was 128.5 and 143.7 cm, respectively. Similarly, the monogerm lines observed by Michalik and Kozak (1993) were one of the highest among the studied genotypes of red beet, although, generally the breeding lines, were shorter than the cultivars, but the height of seed plants ranged from 58 to 119 cm.

The seed plants grown at the distance of 50×40 cm formed 46% more shoots per plant than the plants cultivated at the distance of 50×25 cm (Table 2). Likewise, Balan and Zagorodnii (1986) as well Hemayati *et al.* (2008) noted the rise in branching of sugar beet seed stalks with the increase in planting density. In both years of the present experiment, the plants produced similar number of shoots per plant, while a large variation between the tested genotypes in terms of this trait, was noted. The cultivars formed from 3.4 to 8.4 shoots per plant, in turn the breeding lines produced between 8.5 and 11.4 shoots per plant, except for the lines 357 A and 357 B that formed about 5 shoots per plant. The monogerm lines AR79 A and AR79 B producing over 11 shoots per plant presented one of the strongest branching powers, but unluckily they showed also a strong tendency towards late maturing.

The increase in seed-bearing plants interspacing from 50×25 to 50×40 cm led to the decrease in percentage of single seed stalks from 26.8 to 12.2%, but caused also the increase in percentage of bush plants from 36.2 to 44.7% and bush with main stem seed stalks from 37.0 to 43.2% (Table 2). In the year 2010, the single seed stalks were produced by almost 30% of the stecklings, while in the year 2011 – by only about 10% of the stecklings. In the year 2011, the bush seed-bearing plants were formed by over 54% of the stecklings that was twice more than in the year 2010. The bush with main stem plants were produced by almost 45% of the stecklings in the year 2010 and by about 35% of the stecklings in the year 2011. The tested cultivars formed from about 12 to 43% of the single seed stalks, while breeding lines – from 0 to 35%. The stecklings of monogerm lines AR79 A and AR79 B produced only 0 and 5% of the single seed stalks, respectively. The bush type of seed-bearing plants was formed by about 8 to 87% of the breeding line stecklings and by about 17 to 53% of the cultivar stecklings. The highest number of the bush seed stalks was observed for the line AR79 A (86.7%) that produced only about 13% of the bush with main stem seed-bearing plants. The line AR79 B formed twice less than the line AR79 A of the bush seed stalks and almost 52% of the bush with main stem plants. The percentage of bush with main stem seed stalks ranged from about 27 to 53% for the cultivars and from about 13 to 82% for the breeding lines. Michalik and Kozak (1993) recorded about 57% of the bush seed stalks of red beet and the rest of the plants were bush type with main stem. According to the Janas and Grzesik (2007), the seed-bearing plants architecture greatly affects the yield and quality of seeds, in such a way that the single seed stalks produce good quality seeds, but low yield, instead very bushy seed plants form high yield, but relatively low quality seeds, caused by an uneven ripening of the fruits.

Table 2. Effect of planting density of red beet stecklings on seed plants morphological structure

Treatment	Seed stalk height (cm)	Number of shoots per plant	Seed stalk type (%)		
			single	bush	bush with main stem
Genotype (G)					
218 A	108.5 f	10.23 abc	13.3 de	55.0 bc	31.7 fg
218 B	105.3 fgh	10.28 abc	10.0 def	66.7 b	23.3 ghi
279 mono A	106.7 fg	9.78 bcd	13.3 de	8.3 j	78.4 a
279 mono B	107.9 f	10.13 abc	18.3 d	31.7 fgh	50.0 bcd
357 A	102.2 ghi	6.07 i	35.0 ab	40.0 def	25.0 ghi
357 B	108.2 f	4.78 i	35.0 ab	48.3 cde	16.7 hi
391 A	97.2 j	9.08 cde	20.0 cd	51.7 cd	28.3 fgh
391 B	99.6 ij	8.45 de	30.0 bc	33.3 fg	36.7 d-g
AR79 A	134.8 b	11.28 a	0.0 f	86.7 a	13.3 i
AR79 B	128.5 c	11.42 a	5.0 ef	43.3 c-f	51.7 bc
Astar F ₁	121.8 d	6.13 hi	30.0 bc	36.7 efg	33.3 efg
Modana	118.4 de	8.37 ef	11.7 de	53.3 cd	35.0 efg
Moneta	122.0 d	3.37 j	43.3 a	30.0 f-i	26.7 f-i
Monika	115.8 e	7.83 efg	16.7 d	36.6 efg	46.7 b-e
Monorubra	127.8 c	6.67 gh	13.3 de	51.7 cd	35.0 efg
Okragły Ciemnoczerwony	143.7 a	7.02 fgh	30.0 bc	16.7 ij	53.3 bc
Patryk	119.7 de	6.67 gh	18.3 d	40.0 def	41.7 c-f
Polglob F ₁	122.2 d	6.70 gh	31.7 b	35.0 efg	33.3 efg
W411 A	101.4 hij	9.95 abc	0.0 f	18.3 hij	81.7 a
W411 B	103.1 ghi	10.95 ab	15.0 de	25.0 ghi	60.0 b
Years (Y)					
2010	120.2 a	8.37 NS	28.5 a	26.7 b	44.8 a
2011	109.3 b	8.05 NS	10.5 b	54.2 a	35.3 b
Planting density (D)					
50 × 25 cm	116.6 a	5.21 b	26.8 a	36.2 b	37.0 b
50 × 40 cm	112.8 b	11.21 a	12.2 b	44.7 a	43.2 a
LSD _{0.05} for:					
Y × G	6.0	1.82	14.7	17.6	18.0
G × D	6.0	1.82	14.7	17.6	18.0
D × Y	1.9	0.58	4.6	5.6	5.7
Y × G × D	8.4	2.57	20.8	24.9	25.5

Means in columns followed by the same letter are not significantly different at $P < 0.05$

NS – not significant at $P < 0.05$

The decrease in plants interspacing radically decreased the clusters yield per plant, which for the planting density of 50×25 cm was more than twice lower, comparing to the planting density of 50×40 cm (Table 3). The increase in cluster yield with the increase in plants interspacing was also reported for sugar beet by Nicolau (1978), Balan and Zagorodnii (1986), Podlaski (1987), Leibovich *et al.* (1993) and Hemayati *et al.* (2008) as well for monogerm forage beet by Bordei and Tapus (1981). On the other hand, Kaw and Mir (1975) for the sugar beet seed-bearing plants grown in the narrow rows observed the increase in seed yield. The yield of clusters harvested in the year 2011 was 50.91 g per plant that was notably more than in the year 2010. Generally, the cultivars producing between 42.21 and 69.37 g of clusters per plant, yielded better than the breeding lines, which cluster yield ranged from 29.87 to 47.69 g per plant. The monogerm lines AR79 A and AR79 B, with the clusters yield of 38.50 and 34.34 g per plant, respectively, were among the medium-yielding genotypes, what could partially be caused by a large number of late-maturing shoots formed by these genotypes. Michalik and Kozak (1993) also observed lower fertility of the lines than the cultivars, but there was found a large variation between the genotypes in yield (from 8 to 93 g per plant), where the monogerm lines produced relatively low yield of clusters (about 24 g per plant).

Table 3. Effect of planting density of red beet stecklings on the yield and qualitative characteristics of clusters

Treatment	Yield of clusters (g per plant)	Thousand-seed weight (g)	Mean germina- tion time (day)	Germination capacity (%)
Genotype (G)				
218 A	33.31 fg	25.52 a	3.898 a	96.4 b
218 B	29.87 gh	23.74 c	3.942 a	96.2 b
279 mono A	45.65 cde	14.31 k	4.098 b	89.9 d
279 mono B	38.97 efg	15.63 hi	4.133 b	89.5 d
357 A	44.14 cde	20.08 e	4.128 b	85.5 fg
357 B	39.25 d-g	19.34 f	4.169 b	85.4 fg
391 A	41.96 c-f	19.01 f	3.923 a	95.0 bc
391 B	43.57 cde	18.24 g	3.929 a	93.9 c
AR79 A	38.50 efg	13.37 l	4.284 c	82.6 h
AR79 B	34.34 fgh	13.08 l	4.318 c	84.2 gh
Astar F ₁	54.67 b	21.46 d	3.890 a	86.9 ef
Modana	49.53 bc	15.97 h	4.100 b	83.6 gh

Treatment	Yield of clusters (g per plant)	Thousand-seed weight (g)	Mean germina- tion time (day)	Germination capacity (%)
Moneta	49.87 bc	15.26 hi	4.153 b	96.3 b
Monika	44.11 cde	12.12 m	4.118 b	88.1 de
Monorubra	42.21 c-f	14.31 k	3.866 a	82.3 h
Okągły Ciemnoczerwony	69.37 a	24.52 b	3.897 a	99.2 a
Patryk	49.63 bc	15.13 ij	3.909 a	94.5 bc
Polglob F ₁	48.83 bc	25.16 ab	3.867 a	96.4 b
W411 A	45.60 cde	14.47 jk	4.393 c	75.6 i
W411 B	47.69 bcd	14.23 k	4.555 d	76.2 i
Year (Y)				
2010	38.19 b	17.70 NS	4.429 b	87.6 b
2011	50.91 a	17.80 NS	3.728 a	90.1 a
Planting density (D)				
50 × 25 cm	29.29 b	15.12 b	4.093 NS	87.9 b
50 × 40 cm	59.81 a	20.37 a	4.064 NS	89.9 a
LSD _{0.05} for:				
Y × G	10.37	1.03	0.145	2.59
G × D	10.37	1.03	0.145	2.59
D × Y	3.28	0.33	NS	0.82
Y × G × D	14.67	1.45	0.206	3.67

Explanations: see Table 2

The results of the study indicate that the increase in planting density led to the decrease in cluster size (Table 3). The thousand-seed weight of the plants grown at the spacing of 50 × 40 cm was 20.37 g, but when the plant interspacing was 50 × 25 cm the thousand-seed weight was only 15.12 g. The increase in sugar beet clusters size with the decrease in planting density was previously recorded by Slavov *et al.* (1997), Pospisil and Mustapic (1999) and Hemayati *et al.* (2008). Against, according to Lachowski and Howwicki (1973) the sugar beet plants interspacing did not affect the seed size. The thousand-seed weight of the presently tested genotypes was similar in both years of the experiment. Generally, the monogerm genotypes produced significantly smaller clusters than the multigerm ones. The thousand-seed weight ranged from 12.12 to 15.97 g for the monogerm lines and cultivars, and from 18.24 to 25.52 g for the multigerm genotypes. Michalik (2000) suggested that the thousand-seed weight should be about 11 g for the monogerm and about 20 g for the multigerm red beet clusters.

Dormancy mechanisms of the red beet seeds are relatively strong and caused slow and asynchronous germination (Lee *et al.* 2004). According to Taylor *et al.* (2003), the main factors negatively influenced the germination of beet seeds are: the mucilaginous layer that surrounds the clusters, the ovary cap tenacity of the clusters, and the phenolic germination inhibitors. In the current study, the germination parameters were tested after six months of the storage and before starting the germination test the clusters were rinsed in water following the ISTA (2011) guidelines, which possibly resulted in the satisfactory values of germination characteristics. The planting density applied in the present experiment did not influence the MGT of the clusters. While Slavov *et al.* (1997) and Hemayati *et al.* (2008) observed the increase in germination rate and uniformity with the decrease in planting density. On the contrary, Scott and Longden (1973) the increase in germination vigour noted for the clusters collected from the seed bearing plants cultivated in the narrow rows. In the year 2011, the seeds germinated much quicker than in the year 2010. The lines and cultivars noticeably differed in the rate of germination. In the group of the quickest germinated genotypes, the value of MGT varied between 3.866 and 3.942 days. The slowest germination was observed for the clusters of the monogerm lines AR79 A, AR79 B, W411 A and W411 B, for which the MGT ranged from 4.284 to 4.555 days. The seed-bearing plants cultivated at the spacing of 50 × 40 cm produced the seeds with the GC by two percentage points higher than the plants grown at the spacing of 50 × 25 cm. In the year 2011, the GC was by 2.5 percentage points higher than in the year 2010. Michalik (1993) reported a strong dependence between the red beet genotype and the seed quality. Also currently tested genotypes differed in the final percentage of normal seedlings. The highest GC of 99.2% was recorded in the cultivar Okrągły Ciemnoczerwony. The seeds of the lines W411 A and W411 B presented the lowest GC of 76.2 and 75.6%, respectively. Among the genotypes with relatively low GC of 82.6 and 84.2%, also the monogerm lines AR79 A and AR79 B, respectively, were noted.

The presently reported data has expanded the knowledge about the generative growth period of red beet, including monogerm and multigerm breeding lines, and cultivars. The research has shown that both the planting density of stecklings as well as the genotype affects the seed-bearing plant's morphological structure, the yield of clusters and the quality of seeds. The influence of weather conditions on the seed stalks characteristics also was noted. The new red beet monogerm breeding lines AR79 A and AR79 B, on which special attention was paid in this study, yielded on the average level producing relatively low-quality seeds that was possibly the result of a late-ripening of seed-bearing plants, which showed one of the strongest branching powers, as compared to other genotypes. The results presented in this paper will facilitate Polish breeding programs aimed at the selection of new monogerm red beet lines for hybrid performance.

CONCLUSIONS

1. The increase in red beet plants interspacing from 50×25 to 50×40 cm led to the lower height, but twice higher number of shoots per plant, lower number of single, but higher number of bush and bush with main stem seed stalks, twice higher yield per plant as well higher thousand-seed weight and germination capacity.
2. The height of plants and the type of seed stalks as well as the yield, the mean germination time and the germination capacity depended significantly on the weather conditions, especially, the rainfall totals, during the growing period in different years of the study.
3. A large variation between studied genotypes, including breeding lines and cultivars, in the features of seed-bearing plants, such as morphological structure, clusters yield and seed quality, were noted.
4. Compared to other genotypes, the new monogerm breeding lines AR79 A and AR79 B formed very high, mostly bush or bush with main stem seed stalks, developed one of the greatest numbers of shoots, yielded on the average level and produced relatively low-quality seeds.

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