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SEED DORMANCY AND CONE AND SEED MORPHOLOGY OF SYRIAN JUNIPER (*JUNIPERUS DRUPACEA* LABILL.) IN THE EASTERN MEDITERRANEAN REGION OF TURKEY

DORMANTNOST SJEMENA I MORFOLOGIJA BOBULJASTIH ČEŠERA I SJEMENA KOŠTUNIČAVE BOROVICE (*JUNIPERUS DRUPACEA* Labill.) U ISTOČNO MEDITERANSKOM PODRUČJU TURSKE

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Summary

Syrian juniper, *Juniperus drupacea* Labill., is an Eastern Mediterranean tree species which belongs to the Cupressaceae family and Juniperoidae sub-family. The current geographical range of *J. drupacea* covers the southern parts of the Peloponnese in Greece, the southern parts of Asia Minor and the mountains of Syria and Lebanon. The main part of the species range in Turkey is divided into several centres the most important being located in the Taurus, Anti-Taurus and Amanos mountains. It is a dioecious tree, with conical crown, that reaches 10-20 (40) m in height. The fleshy cones are ovoid to globose, 20-25 mm in diameter, brownish-purple or bluish-black, glaucosus and pruniose when ripe in the second year. They mostly have 3 seeds forming a characteristic drupe-like stone. The natural regeneration of Syrian juniper is very difficult because of animal consumption, grazing and the united seeds into the woody structure. This study was carried out to determine the seed and cone morphology and seed physiology of Syrian juniper, *Juniperus drupacea* Labill., from three provenances (Kahramanmaraş, Mersin, Adana) in the Eastern Mediterranean region of Turkey. In total 11 morphological traits were measured. The average cone length, cone diameter, and cone weight were 22.49 mm, 20.86 mm and 4.659 g, respectively. The average seed length, width, thickness, weight and pulp weight were 10.07 mm, 3.30 mm, 2.77 mm 0.045 g and 2.220 g in order. After morphological analysis, a series of experiments was conducted to identify the best stratification treatment for breaking dormancy in Syrian juniper seeds. The highest germination percentage was obtained after warm and cold stratification (80.7%). The seeds prechilled 8 weeks and soaked 500 ppm GA_3 also demonstrated high germination percentage. The current study demonstrated that *J. drupacea* seeds has morphophysiological dormancy.

KEY WORDS: *Juniperus drupacea*, cone and seed morphology, seed dormancy

INTRODUCTION
UVOD

Syrian juniper, *Juniperus drupacea* Labill., is an Eastern Mediterranean tree species which belongs to the Cupressaceae family and Juniperoidae sub-family. The current geographical range of *J. drupacea* covers the southern parts of the Peloponnese in Greece, the southern parts of Asia Minor and the mountains of Syria and Lebanon. The main part

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of the species range in Turkey is divided into several centres the most important being located in the Taurus, Anti-Taurus and Amanos mountains. It usually occurs at 600-1800 (2050) m altitude on rocky and lime terrains in Antalya, Karaman, Konya, Mersin, Adana, Hatay, Osmaniye and Kahramanmaraş. Mostly forms mixed stands with Crimean pine (*Pinus nigra* J.F.Arnold subsp. *pallasiana* /Lamb./ Holmboe), Turkish pine (*Pinus brutia* Ten), cedar of Lebanon (*Cedrus libani* A. Rich.), Taurus fir (*Abies cilicica* /Antoine et Kotschy/ Carrière), oaks (*Quercus*L spp.) and junipers (*Juniperus*L. spp.). It is a dioecious tree, with conical crown, that reaches 10-20 (40) m in height. Bark is brown and cinnamon beneath, exfoliating in narrowing strips. Leaves are acicular, 10-25 mm long and 3-4 mm wide, with two white bands on the above. Needles are arranged in alternate whorls of three. The seed cones of Syrian juniper are the largest of any juniper species. They are ovoid to globose, 20-25 mm in diameter, brownish-purple or bluish-black, glaucosus and pruniose when ripe in the second year. The fleshy cones mostly have 3 seeds forming a characteristic drupe-like stone (Vidaković, 1991; Adams, 2014; Sobierajska *et al.,* 2016). Likewise, the seeds are very attractive food source for animals. The natural regeneration of Syrian juniper is very difficult because of animal consumption, grazing and the united seeds into the woody structure. In nature, seeds can germinate in period of 3-5 years (Browicz, 1982; Yaltırık, 1988; Gültekin, 2007; Gültekin *et al.* 2004). In the south of Turkey, *J. drupacea* cones are traditionally used in molasses processing.

Morphological data are still very important in many fields of plant sciences (Kaplan, 2001; Douaihy *et al*., 2012; Poljak *et al*., 2015): different taxon delimitation (Sękiewicz *et al*., 2016), population variability (Brus *et al.* 2011, 2016; Douaihy *et al*., 2012),cultivar characterisation (Poljak *et al*., 2016) and selection (Polat and Özkaya, 2005), as well as in morphological and physiological seed characterisation (Powell, 2010; Yilmaz and Yüksel, 2014; Drvodelić *et al*., 2015; Daneshvar *et al*., 2016). In fact, according to the Powell (2010) in the past few years there is an increased interest for seed science, i.e morphological and physiological characteristics of seeds and their capacity to germinate and survive.

Seeds must be alive and matured as physiological and morphological for germination. Some alive seeds can't germinate despite they have optimal conditions (moisture, temperature, oxygen light/dark). This condition is named as seed dormancy (Schmidt, 2000; Bewley and Black 2013). Seed dormancy seperated into 5 basic classes. Physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy and combinational (physical and physiological) dormancy (Baskin and Baskin, 2004; Bonner, 2008). Juniperus species seeds show different dormancy characteristics like physiological dormancy (*J. phoenicea*) or morphophysiological dormancy (*J. polycarpos*) so it is difficult to propogate from seeds (Broom, 2003; Daneshvar *et al*. 2016; Al-Ramamneh *et al*. 2012).

The aim of this study was to determine seed and cone morphology, and to search the dormancy depth and level and proper dormancy-breaking pre-treatments in Syrian juniper seeds from three provenances in the eastern Mediterranean region of Turkey. The effect of GA_3 hormone on the germination behaviour of seeds was also tested.

MATERIAL AND METHODS
MATERIJALI I METODE

The cones were collected from three provenances (Mersin-Mut, Kahramanmaraş-Andırın, Adana-Kozan) in Turkey in February and March 2014 (Table 1). The seeds, for dormancy treatments and exogenous application of hormones, were extracted from the cones by hammering the cones, and then they were air dried to 5.4 MC at ambient temperature for two days. The air dried seeds were stored in the closed bottles in the refrigerator till use.

A Syrian juniper cone is composed of three parts. The pulp carpel, woody carpel and seed. From each provenance 50 cones were randomly selected and measured. Firstly cone traits (cone length, cone diameter, cone weight) were measured. Then, pulp part was removed and woody carpel was measured (length, diameter and weight). Finally woody part was broken, the seeds were extracted and the number of seeds and seed weight were determined. In this way, the weight percentage of pulp part, woody part, and seed part in a cone were identified. Furthermore, 50 seeds from each provenance were randomly chosen seed length, width and thickness were measured. 1000-seed weight was also found from 800 seeds (8*100) according to the ISTA rules (1996).

To found out the dormancy level and pre-treatment requirements, seeds from three different provenances were subjected 0 (control), 4 (4w), 8 (8w), 12 (12w) and 16 (16w)

Table 1. Study area.
Tablica 1. Područje istraživanja

Provenances *Provenijencija*	Latitude *Geog rafska širina*	Longitude *Geog rafska dužina*	Altitude *Nadmorska visina* (m)
Mersin-Mut	36°40′	33°44′	1116
Kahramanmaraş-Andırın	37°45′	36°42′	943
Adana-Kozan	37°38′	35°50′	895

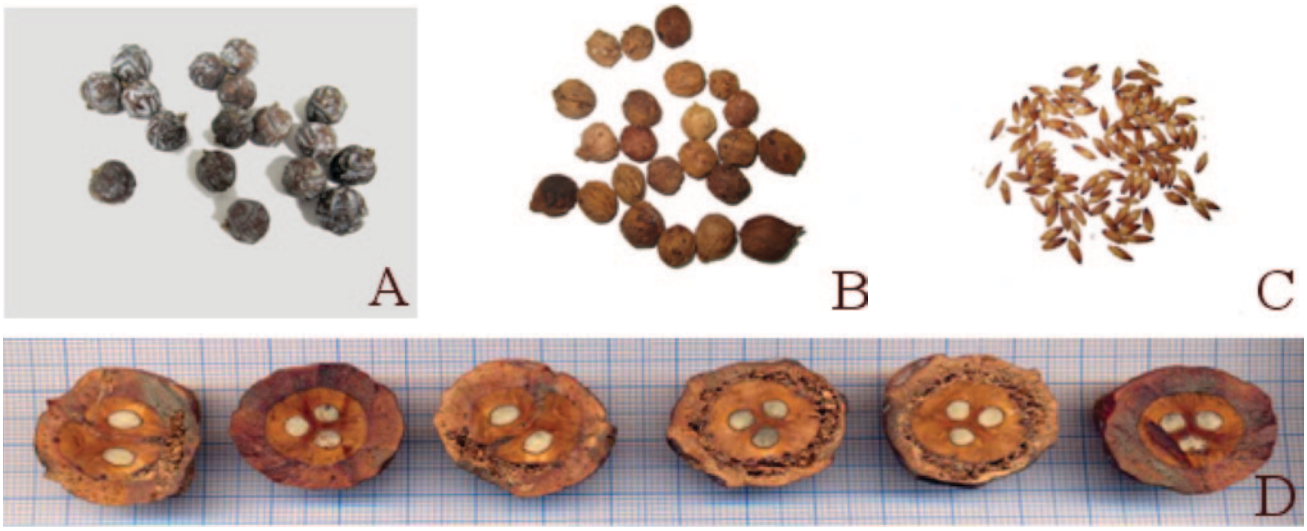

Figure 1. (A) cones; (B) woody part of the cones; (C) seeds; and (D) cross section of Syrian juniper cones.
Slika 1. (A) bobuljasti češeri; (B) drvenasti dijelovi bobuljastih češera; (C) sjeme; i (D) bobuljasti češeri koštuničaste borovice na poprečnom presjeku.

weeks pre-chilling at 4 °C, as well as the 4 weeks warm incubation at 12 °C subsequently 9 weeks cold stratification at 4 °C (4wW+9w).

Germination tests were done in 11 cm diameter Petri dishes on two layer of filter paper moistened with distilled water with 150 seeds (3×50) at 12 °C. The seeds were checked every 4 days and the germinated seeds were recorded and removed. Germination tests were continued for 92 days. The seeds were removed from Petri dishes after their radicles were elongated for 3 mm.

To investigate the effects of GA_3 on the dormancy and the germination, seeds from two different provenances (Adana and Mersin) were tested. Two types of seed were used, non–pre-chilled and 8 weeks pre-chilled. Both types of seed were subjected to different GA_3 hormone doses (100 ppm and 500 ppm) for 24 hours.

Germination percentage (GP) and mean germination time (MGT) were calculated according to following formulas (Bewley *et al*., 2013):

$$GP(\%)=(\Sigma n_i/N)\times 100$$

where GP is the germination percentage, n_i is the number of germinated seeds at week i, and N is the total number of incubated seeds per tests; and

$$MGT=\Sigma(t_i.n_i)/\Sigma n_i$$

where MGT is the mean germination time, $t_i$is the number of weeks from the beginning of the test, and n_i is the number of germinated seeds recorded on week t_i.

Morphological characteristics of cones and seeds, germination percentage as well as mean germination time were analyzed using ANOVA. The percent values (GP) were transformed using arc sin square root before variance analyses.

RESULTS
REZULTATI

The average cone length, cone diameter and cone weight were 22.49 mm, 20.86 mm and 4.659 g respectively. There were no significant differences between provenances in terms of cone length and cone diameter. Adana provenance had the heaviest cone in average. Average woody part length, diameter and weight were 17.62 mm, 14.76 mm and 2.34 g in order. The average pulp weight was 2.22 g. The average number of seeds were 2.2 and the average seeds weight 0.097 g. Average woody part, pulp part and seed weight percentage in a cone were 50.69%, 47.18% and 2.12% respectively.

Average 1000-seed weight for three provenances was 44.66 g at about 5.4%MC. The average seed length, width, thickness and weight were 10.07 mm, 3.30 mm, 2.77 mm and 0.045 g respectively. There were no significant differences between the provenances in terms of seed width and thickness. Kahramanmaraş provenance had the heaviest seed in average (Table 2).

Pre-chilling applications of 4 w and 8 w were inadequate for breaking the dormancy. By contrast, 12 w and 16 w pre-chilling applications increased the germination percentages, but these treatments didn't eliminate the dormancy completely. 4 week warm stratification subsequently with 9 w pre-chilling resulted in the highest germination percentage. After the 0-w, 4-w, 8-w, 12-w, 16-w, and 4w warm stratification +9 w pre-chilling average germination percentages were 6%, 15.3%, 14.2%, 26.9%,27.8%, 65.3% respectively (Table3). Mean germination time of seeds after different applications at germination temperature at 12 °C is presented in Table 4.

Gibberellic acid was applied to 8-w pre-chilled and non-pre-chilled seeds which were collected from Adana and Mersin provenance. The hormone didn't increase germination percentage innon-pre-chilled seeds. On the contrary, this hor-

Table 2. Morphological characteristics of J. drupacea seeds and cones.
Tablica 2. Morfološke značajke bobuljastih češera i sjemena J. drupacea.

Provenance *Provenijencija*	Cone *Bobuljasti-češer*			Woody part of the cone *Drvenasti dio bobuljastog češera*			Seed *Sjeme*				Fleshy part of the cone Mesnati dio bobuljastog češera
	Length *Dužina* (mm)	Width *Širina* (mm)	Weight *Težina* (g)	Length *Dužina* (mm)	Width *Sirina* (mm)	Weight *Težina* (g)	Length *Dužina* (mm)	Width *Širina* (mm)	Thickness *Debljina* (mm)	Weight *Težina* (g)	Weight Težina (g)
Maraş	22.42 a[1]	20.58 a	4.648 ab	17.71 a	15.20 a	2.485 a	10.30 a	3.33 a	2.81 a	0.048 a	2.047 b
Adana	22.82 a	20.91 a	4.861 a	17.58 a	14.88 a	2.412 a	10.21 a	3.25 a	2.74 a	0.042 b	2.355 a
Mersin	22.23 a	21.08 a	4.469 b	17.57 a	14.20 b	2.131 b	9.69 b	3.33 a	2.76 a	0.044 b	2.256 ab
Average – *Prosječno*	22.49	20.86	4.659	17.62	14.76	2.342	10.07	3.30	2.77	0.045	2.220

[1] The values on the same column followed by the same letters are not significantly different ($p<0.05$).
[1] *Vrijednosti u istoj koloni označene istim slovom ne razlikuju se na razini signifikantnosti od ($p< 0.05$).*

Table 3. The germination percentages after various pre-treatments at 12 °C.
Tabela 3. Postotak klijavosti pri temperaturi od12 °C nakon različite predsjetvene pripreme.

Provenance *Provenijencija*	Pre-chilling duration *Trajanje stratifikacije*						Average *Prosječno*
	0 (control) *kontrola*	4 weeks *4 tjedna*	8 weeks *8 tjedana*	12 weeks *12 tjedana*	16 weeks *16 tjedana*	4 w WS+9 w	
Adana	8.7 d[1]	16.0 c	11.3 cd	13.3 cd	22.7 b	45.3 a	19.6 C[2]
Mersin	9.3 e	19.3 d	19.3 d	44.0 b	28.0 c	70.0 a	31.7 A
Maraş	0.0 e	10.7 d	12.0 d	23.3 c	32.7 b	80.7 a	26.6 B
Average – *Prosječno*	6.0 d	15.3 c	14.2 c	26.9 b	27.8 b	65.3 a	

[1] The values on the same line followed by the same letters are not significantly different ($p<0.05$).
[1] *Vrijednosti u istom retku označene istim malim slovom ne razlikuju se na razini signifikantnosti od ($p< 0.05$).*
[2] The values on the same column followed by the same capital letters are not significantly different ($p<0.05$).
[2] *Vrijednosti u istoj koloni označene istim velikim slovom ne razlikuju se na razini signifikantnosti od ($p< 0.05$).*

Table 4. Mean germination time of seeds after different applications at germination temperature at 12 °C.
Tablica 5. Srednje vrijeme klijanja sjemena J.drupacea pri temperaturi od 12 °C nakon različite predsjetvene pripreme.

Provenance Provenijencija	Pre-chilling duration *Trajanje stratifikacije*						Average *Prosječno*
	0 (control) *kontrola*	4 weeks *4 tjedna*	8 weeks *8 tjedana*	12 weeks 12 *tjedana*	16 weeks *16 tjedana*	4 w WS+9 w	
Adana	78.0 d[1]	74.4 d	54.5 c	47.1 b	42.4 b	23.7 a	53.4 C[2]
Mersin	63.9 d	53.6 c	55.2 c	41.4 b	41.9 b	19.1 a	45.9 B
Maraş	-	56.1 d	53.5 d	37.5 c	29.2 b	14.0 a	38.1 A
Average – *Prosječno*	70.9 f	61.4 e	54.4 d	42.0 c	37.8 b	18.9 a	

[1] The values on the same line followed by the same letters are not significantly different ($p<0.05$).
[1] *Vrijednosti u istom retku označene istim malim slovom ne razlikuju se na razini signifikantnosti od ($p< 0.05$).*
[2] The values on the same column followed by the same capital letters are not significantly different ($p<0.05$).
[2] *Vrijednosti u istoj koloni označene istim velikim slovom ne razlikuju se na razini signifikantnosti od ($p< 0.05$).*

mone promoted the germination in 8-w pre-chilled seeds and increased the germination percentage. There was a distinctive difference between the germination percentage for the 8-w pre-chilled and non-pre-chilled seeds (Figure 2).

DISCUSSION AND CONCLUSIONS
RASPRAVA I ZAKLJUČCI

The seed cones of Syrian juniper are the largest of any juniper species(Yaltırık, 1988). In present study, the average cone length and diameter was found 22.49, 20.86 mm, respectively. In another study (Sobierajska *et. al.*, 2016), average cone length and cone diameter of *J. drupacea* for 12 populations was found 20.54 and 19.18 mm, which were relatively similar to our results. The average number of seeds extracted from the cones was 2.2. There wasn't a statistical difference between the provenances in terms of cone length, diameter and seed number. In other two studies the average seed number was 2.1 (Gültekin *et al.*, 2005) and 2.3 (Gürlevik and Gültekin, 2008).

In this study the average 1000-seed weight was found 44.66 g for three provenances.1000-seed weight generally occurs between 10-90 g depending on the species and provenances (Bonner, 2008). In *J. virginiana* L. and *J.occidentalis*

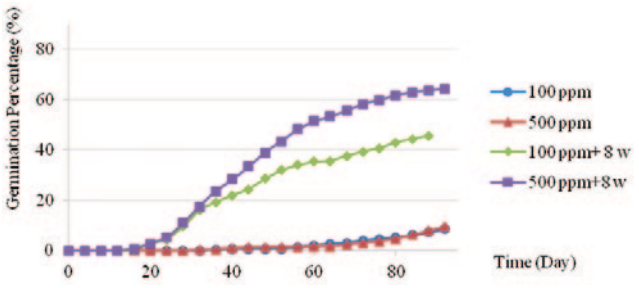

Figure 2. Germination curve of *J. drupacea* seeds exposed to GA_3 hormone.

Slika 2. Krivulje klijavosti sjemena J. drupacea na koje je primjenjen hormon GA_3.

Hook.1000-seed weight was 10.4 and 36.9 g respectively (Bonner, 2008).The average seed length, width, thickness and weight were found 10.07 mm, 3.30 mm, 2.77 mm and 0.045 g, in order.

There were significant differences between the pre-treatments with regard to germination percentages. The seeds without any pre-treatments showed very low germination percentage (6%). But after different pre-treatment's germination percentage was increased. This showed that *J. drupacea* seeds have deep dormancy. The applied pre-chilling treatments didn't entirely eliminate the dormancy. However the seeds for three provenances after 4 weeks warm stratification and subsequently 9 weeks pre-chilling were very effective on the elimination of dormancy and they increased the germination percentage (average 65.3%). Barbour and Carvalho (2009) tried to germinate *J. scopulorum* Sarg. seeds with 21 different pre-treatment's. In that study they obtained the best results (55%) with 16 weeks of warm stratification and 13 weeks cold stratification. For the above mentioned treatments they soak the seeds 3 days in water.

Morphological and physiological seed dormancy is well recorded in many woody species: *Fraxinus excelsior* L. (Suszkaet *al.*, 1996), *Carpinus betulus* L., *Tilia cordata* Mill., *Juniperus communis* L., *Cornus mas* L. (Gosling, 2007), *Taxus chinensis* (Pilg.) Rehder var. *mairei* (Lemée ex H. Lév.) W. C. Cheng et L. K. Fu (Chien *et al.*, 1998) *Juniperus excelsa* M. Bieb.var. *polycarpos* (K. Koch) Takht. (Daneshvar, 2015), *Prunus campanulata* Maxim. (Chien *et al.*, 2002), *Viburnum lantana* L. (Santiago *et al.*, 2015) etc. To break this dormancy different pre-treatments are necessary, e.g.warm incubation and pre-chilling (Baskin and Baskin, 2004). The best results in our study in terms of mean germination time were achieved with 4 weeks of warm stratification and 9 weeks of pre-chilling.

It is well now that gibberellic acid breaks the dormancy, promotes germination and inhibits ABA effect in germination processes (Bewley and Black, 1994; Kucera *et al.*, 2005). In our study non-pre-chilled seeds that were treated with 100 ppm and 500 ppm GA_3showed very low germination percentages. On the other hand, 8 weeks pre-chilled seeds which were subjected to GA_3 hormone reached the highest germination percentage (64.3%). Gibberellic acid without any pre-treatments didn't have a significant effect on the germination of *J. drupacea* seeds.

J. drupacea is a decorative, evergreen tree with high ornamental potential. Considering that it could be used in parks and gardens as an ornamental species. Furthermore, *J. drupacea* seedlings should be produced in forest nurseries and used in reforestation programs.

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Sažetak

Sirijska smreka, *Juniperus drupacea* Labill. je istočno mediteranska vrsta stabla koja pripadaporodici *Cupressaceae*, rodu*Juniperoidae*. *J. drupacea* trenutno pokriva geografsko područje južnog dijela Peloponeza u Grčkoj, južne dijelove Male Azije te sirijske i libanonske planine. Glavni dio područja te vrste u Turskoj podijeljen je na nekoliko središta, od kojih je najvažniji smješten u Taurskom gorju, Anti-Taurus gorju te planinama Amanos. Stablo je diecično, s konusnom krošnjom koja može doseći 10-20 (40) m visine. Mesnati češeri su ovalnog do okruglog oblika, 20-25 mm u promjeru, smećkasto-ljubičaste ili plavkasto-crne boje, te žućkasti i bjelkasti kada su zreli tijekom druge godine. Uglavnom imaju 3 sjemenke koje tvore karakterističnu košticu nalik na košticu koštičavog voća. Prirodna regeneracija sirijske smreke vrlo je teška zbog konzumacije od strane životinja, ispaše i sjemenki koje su objedinjene u drvenastu strukturu. Ovo je istraživanje provedeno kako bi se odredila morfologija sjemenke i češera te fiziologija sjemenke sirijske smreke, *Juniperus drupacea* Labill., koja potječe iz tri provincije (Kahramanmaraş, Mersin, Adana) u istočnomediteranskoj regiji Turske. Ukupno je izmjereno 11 morfoloških obilježja. Prosječna duljina češera bila je 22,49 mm, promjer češera 20,86 mm, a težina češera 4,659 g. Prosječna duljina, širina, debljina, težina sjemenke i težina pulpe sjemenke iznosile su redom 10,07 mm, 3,30 mm, 2,77 mm, 0,045 g i 2,220 g. Nakon morfološke analize, proveden je niz eksperimenata kako bi se odredila najbolja mjera stratifikacije radi prekidanja dormantnosti sjemenki sirijske smreke. Najveći postotak klijavosti dobiven je nakon tople i hladne stratifikacije (80,7%). Sjemenke koje su se prethodno hladile 8 tjedana te su namakane u 500 ppm GA_3također su pokazale velik postotak klijavosti. Ovo je istraživanje također pokazalo da sjeme*J. drupacea*posjeduje morfološku dormantnost.

KLJUČNE RIJEČI: *Juniperus drupacea*, morfologija češera i sjemena, dormantnost sjemena