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Relationship Between Agroclimatic Conditions and Seed Quality and Seedling Growth of Crimson Clover

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ABSTRACT

This study investigated the relationship between seed quality parameters (germination, dormancy, initial shoot and root growth) of crimson clover originating from six locations in eastern Serbia and the agroclimatic conditions over a three-year period. The results indicate significantly higher seed germination rates at temperatures of 20°C and 25 °C compared to 15 °C.

A positive and statistically significant ($p \leq 0.05$) to very strong ($p \leq 0.001$) correlation was observed between mean monthly temperatures in May and seed dormancy.
In contrast, a significant negative ($p \leq 0.05$) to strong ($p \leq 0.01$) correlation was found between total monthly precipitation in May and seed germination. No statistically significant correlations were identified between mean monthly temperatures in March and April and any of the seed quality parameters or seedling growth traits. Similarly, no statistically significant correlation was found between total precipitation in March and April and seed quality indicators.
In light of global warming predictions and rising temperatures, these findings underscore the importance of adopting agronomic practices, such as seed treatment, to mitigate dormancy and increase germinable seed rates.

Keywords: Mean monthly air temperature, Monthly precipitation sum, Seed germination and dormancy, Shoot and root growth

HIGHLIGHTS

- A significant positive to very strong correlation ($p \leq 0.05$ to $p \leq 0.001$) was observed between mean temperatures in May and seed dormancy.
- A significant negative correlation ($p \leq 0.05$ to $p \leq 0.01$) was identified between total precipitation in May and seed germination.
- There was no statistically significant correlation between seed germination or dormancy and either mean monthly temperatures or total precipitation during March and April.

1. INTRODUCTION

The genus *Trifolium* comprises approximately 250 species (Zohari and Heller, 1984), though only a few hold significant economic value as forage crops. White clover (*Trifolium repens* L.) is among the most widely cultivated species of the genus *Trifolium*. It is the most extensively distributed pasture legume across the temperate regions of Europe, western Asia, and northwestern Africa (Deng et al., 2025). Red clover (*Trifolium pratense* L.), a species of Mediterranean origin, has become cosmopolitan and is well-adapted to a wide range of edaphoclimatic conditions (Greveniotis et al., 2025).

Red clover is commonly grown for silage and hay in many temperate regions (Zupanič et al., 2025), and it naturally occurs in grasslands and pastures throughout Europe, western Asia, and northwestern Africa (Egan et al., 2021; Petrović et al., 2014). Grasslands and pastures in the Republic of Serbia account for 28.7% of the total agricultural land (Statistical Office of the Republic of Serbia, 2023), highlighting considerable potential for enhancing forage production. Compared to white clover and red clover, crimson clover (*Trifolium incarnatum*) is considerably less widespread. However, in eastern Serbia, it accounts for approximately 10% of pasture areas (Aćić et al., 2013), and up to 14% of pastures near the towns of Knjaževac and Zaječar (Petrović et al., 2007; Bogosavljević et al., 2007). Crimson clover can be cultivated similarly to other forage legumes (e.g., alfalfa and red clover), and in some regions, it produces satisfactory yields and forage quality (Zamanian et al., 2024; Peker et al., 2020).

Compared to monoculture of forage grasses, legume–grass mixtures—whether in pastures or sown forage systems—have been shown to enhance biological nitrogen fixation (requiring minimal nitrogen fertilization), improve soil quality, and increase both yield and forage nutritive value, particularly through higher protein content (Folina et al., 2025; Tomić et al., 2025). However, Velijević et al. (2018) reported that *Trifolium pratense* exhibits suboptimal germination rates under standard conditions, relative to its optimal sowing requirements, indicating that pre-sowing seed treatment is recommended to enhance germination performance. Similar germination challenges have been reported for *T. repens* (Chu et al., 2022). In *T. incarnatum*, the primary issue is a high proportion of dormant seed (around 50%), which results in lower overall germination of about 35% (Štrbanović et al., 2025). However, appropriate treatments can raise germination to as much as 60% (Štrbanović et al., 2025). Seed germination is influenced not only by genetic factors (Wawo et al., 2024), but also by the environmental conditions prevailing during seed development on the maternal plant (Donohue, 2009). Therefore, temperature and water availability directly impact seed development and subsequent germination (Bailly & Gomez Roldan, 2023). For this reason, investigating seed quality as a response to climate conditions and climate change is a step toward understanding their effects and proposing new agronomic approaches that provide future solutions (Bailly & Gomez Roldan, 2023).

The ability of seeds to overcome dormancy and successfully germinate is critical for all plant species (Willis et al., 2014) and this ability is strongly influenced by agroclimatic factors (Finch-Savage and Bassel, 2016). Despite its importance, there is a lack of studies directly examining the correlation between climatic conditions and seed quality. Crimson clover is a valuable species in eastern Serbia and many other regions globally, yet its germination performance under varying environmental conditions remains underexplored. The aim of this study was to investigate the relationship between agroclimatic conditions and seed quality—specifically germination and seedling growth—of crimson clover over a three-year period across six locations in eastern Serbia.

2. MATERIALS AND METHODS

Plant Material and Meteorological Data

Based on previous studies by Petrović et al. (2007) and Bogosavljević et al. (2007), as well as our own research conducted on natural meadows and pastures across six locations in eastern Serbia, the presence of crimson clover (*Trifolium incarnatum* L.) was confirmed and geographical coordinates were recorded using a GPS device.

Seed harvesting was carried out at the end of May or during the first days of June each year, during 2021-2023 growing seasons (Table 1). Seeds were collected when the stems and inflorescences had turned brown. Inflorescences were collected manually, seeds were extracted by hand, and then naturally dried until seed moisture dropped below 13%. Seeds from all locations (I- VI) were stored in paper bags under ambient conditions in a seed storage facility, as described by Stanisavljević et al. (2020).

Table 1. Geographic origin and collection time of *T. incarnatum* seeds

(Locations)	Latitude (N)	Longitude (E)	m a.s.l	Collection time -Year		
				2021	2022	2023
I (Bor)	43° 54′ 15″	22° 51′ 03″	199	27. 05	21.05	25. 05
II (Zaječar)	43° 59′ 11″	22° 20′ 18″	108	30. 05	29. 05	27. 05
III (Boljevac)	43° 59′ 41″	21° 57′ 14″	270	02. 06	05.06	02. 06
IV (Negotin)	44° 13′ 16″	22° 25′ 04″	223	26. 05	29. 05	29. 06
V (Knjaževac)	43° 39′ 31″	22° 14′ 58″	246	28. 05	01.06	01. 06
VI (Kladovo)	44° 38′ 47″	22° 33′ 17″	48	02. 06	29. 05	28. 06

Identification of *T. incarnatum* was based on morphological characteristics of the stems, leaves, and, particularly, the inflorescences and seeds. Meteorological conditions were monitored during

March, April, and May of 2021, 2022, and 2023, at six locations, in eastern Serbia. The parameters included mean monthly air temperatures (Table 2) and total monthly precipitation (Table 3).

Table 2. Mean monthly air temperatures (T ºC) during three years for months, March, April, and May

Year	Month	Locations (I – VI)						$\overline{X}$			CV
		I	II	III	IV	V	VI		Min.	Max.	%
2021	March	6.2	4.8	3.6	5.6	3.9	5.1	4.9	3.6	6.2	20.37
	April	10.3	9.2	8.2	10.2	8.5	11.2	9.6	8.2	11.2	12.09
	May	15.3	16.1	15.6	17.6	15.9	18.3	16.5	15.3	18.3	7.29
2022	March	4.9	3.7	3.1	5.8	3.6	6.3	4.6	3.1	6.3	28.51
	April	11.4	11.8	11.2	12.2	11.8	13.2	11.9	11.2	13.2	5.96
	May	16.1	17.6	16.4	19.1	17.2	20.3	17.8	16.1	20.3	9.31
2023	March	8.3	8.8	8.1	7.6	7.9	9.1	8.3	7.6	9.1	6.77
	April	10.4	11.4	10.6	10.2	10.8	12.3	11.0	10.2	12.3	7.12
	May	14.9	16.7	14.8	15.2	15.2	17.6	15.7	14.8	17.6	7.28
$\overline{X}$		10.9	11.1	10.2	11.5	10.5	12.6				

Table 3. Average rainfall (mm) during three years for months, March, April, May

Year	Month	Locations (I – VI)									
		I	II	III	IV	V	VI	∑	Min.	Max.	CV %
2021	March	69.3	38.8	62.3	49.8	61.2	55.3	337	38.8	69.3	19.16
	April	59.3	49.8	59.6	56.0	66.3	61.2	352	49.8	66.3	10.38
	May	77.1	34.5	57.8	27.4	56.3	59.9	313	27.4	77.1	34.88
2022	March	61.2	17.4	68.4	70.2	59.3	55.3	332	17.4	70.2	35.07
	April	48.4	33.3	51.2	33.7	52.2	41.6	260	33.3	52.2	19.62
	May	30.5	52.1	34.3	61.3	44.6	56.3	279	30.5	61.3	26.41
2023	March	51.3	29.8	88.3	99.8	48.6	68.6	386	29.8	99.8	40.85
	April	49.6	62.4	61.2	67.9	59.3	51.3	352	49.6	67.9	11.88
	May	72.5	42.1	99.3	41.3	44.6	43.3	343	41.3	99.3	41.67

∑	519	360	582	507	492	493

After 3 to 4 months—coinciding with the autumn sowing season and/or pasture overseeding in Serbian agroclimatic conditions—seed samples were subjected to quality assessments and seedling growth testing.

Seed quality evaluation

Germinated seeds (GS) and **Dormant seeds (DS).** The experimental design comprised 450 seeds, arranged as 50 seeds per replication across three replications for each of three temperature regimes per each location and each year. Germinated and dormant seeds were assessed on the seventh day after placement in germination chambers, under dark conditions at 20 °C, in accordance with ISTA rules (ISTA, 2020). In addition to testing at 20°C, we also tested GS and DS seeds at 15°C and 25°C, to test the correlation between temperature and seed quality parameters.

When seed viability could not be clearly determined, a tetrazolium test was applied, which relies on the activity of certain dehydrogenase enzymes and indirectly measures the ability of living seed tissues to breathe (França-Neto and Krzyzanowski 2022).

Seedling growth

Shoot and root length (in cm), was measured on the same day as germination. This is the first time when the differences in the seedling growth can be observed. Shoot length was measured from the base of the shoot/root junction till the tip of the longest leaf with a gradual scale and expressed in cm. Root length was measured from the base of the shoot/root junction till the tip of the longest root (Stanisavljević et al., 2020; https://www.tropicalgrasslands.info/index.php/tgft/article/view/639/395).

Statistical Analysis

Following ANOVA, Tukey's multiple range test was used to evaluate the statistical significance of treatment effects. Data were subjected to arcsine transformation. Standard error of the mean (SEM) and coefficient of variation (CV%) were calculated to express data variability. Data analysis was conducted using Minitab software, version 16.1.0 (Minitab Inc., State College, Pennsylvania, USA; https://www.minitab.com/en-us/, accessed November 25, 2023), and the R statistical computing environment (R Core Team, 2018), freely available from the R Foundation for Statistical Computing, Vienna, Austria.

3. RESULTS

During the three study years, the influence of location on variability in average monthly temperature was highest in March 2022 and March 2021 (CV=28.51% and CV=20.37%, respectively), and lowest in April 2022 (CV=5.96%) (Table 1).

The highest variability in monthly precipitation by location was observed in May 2023 (CV=41.67%), followed by March 2023 (CV=40.85%), March 2022 (CV=35.07%), and May 2021 (CV=34.88%). The lowest variability was recorded in April 2021 (CV=10.38%) and April 2023 (CV=11.88%) (Table 2).

Seed testing from six locations across all years showed that all locations had significantly higher germination ($p \le 0.05$) at 20 ºC compared to 15 ºC, while no significant difference was observed between germination at 20 ºC and 25 ºC ($p \ge 0.05$) (Table 4).

Table 4. Germination (GS) and dormancy (DS) of seeds among different locations (I-VI) at different temperatures during 2021–2023

Year	Locations	GS in Temperatures ºC				DS in Temperatures ºC			
		15	20	25	$\overline{X}$	15	20	25	$\overline{X}$
2021	I	$28^{\pm0.28\ bB}$	$31^{\pm0.42\ bA}$	$32^{\pm0.61\ bA}$	30.3	$49^{\pm0.55\ bA}$	$50^{\pm0.55\ bA}$	$50^{\pm0.69\ bA}$	49.7
	II	$32^{\pm0.48\ aB}$	$35^{\pm0.48\ aA}$	$35^{\pm0.78\ aA}$	34.0	$50^{\pm0.36\ bA}$	$51^{\pm0.32\ abA}$	$51^{\pm0.36\ bA}$	50.7
	III	$31^{\pm0.36\ aB}$	$35^{\pm0.66\ aA}$	$34^{\pm0.45\ aA}$	33.3	$49^{\pm0.69\ bA}$	$51^{\pm0.48\ abA}$	$50^{\pm0.29\ bA}$	50.0
	IV	$32^{\pm0.71\ aB}$	$35^{\pm0.41\ aA}$	$35^{\pm0.52\ aA}$	34.0	$52^{\pm0.31\ aA}$	$52^{\pm055\ abA}$	$51^{\pm0.81\ bA}$	51.7
	V	$31^{\pm0.56\ aB}$	$34^{\pm0.77\ aA}$	$35^{\pm0.39\ aA}$	33.3	$49^{\pm0.49\ bA}$	$50^{\pm0.67\ bA}$	$50^{\pm0.55\ bA}$	49.7
	VI	$30^{\pm0.39\ abB}$	$33^{\pm0.81\ abA}$	$33^{\pm0.63\ abA}$	32.0	$52^{\pm0.55\ aA}$	$53^{\pm0.37\ aA}$	$53^{\pm0.29\ aA}$	52.7
$\overline{X}$		30.7	33.8	34.0		50.2	51.2	50.8	
2022	I	$35^{\pm0.49\ aA}$	$36^{\pm0.66\ aA}$	$36^{\pm0.29\ aA}$	35.7	$52^{\pm0.62\ bA}$	$53^{\pm0.42\ cA}$	$52^{\pm0.46\ dA}$	52.3
	II	$30^{\pm0.52\ bcB}$	$33^{\pm0.31\ bA}$	$34^{\pm0.32\ abA}$	32.3	$53^{\pm0.21\ bA}$	$55^{\pm0.36\ bA}$	$54^{\pm0.55\ cAB}$	54.0
	III	$34^{\pm0.64\ aB}$	$36^{\pm0.46\ aA}$	$37^{\pm0.68\ aA}$	35.7	$53^{\pm0.48\ bA}$	$52^{\pm0.45\ cA}$	$52^{\pm0.31\ dA}$	52.3

	IV	$31^{\pm0.72\text{ bcA}}$	$32^{\pm0.52\text{abA}}$	$32^{\pm0.55\text{ bA}}$	31.7	$56^{\pm0.61\text{ aAB}}$	$57^{\pm0.33\text{ aAB}}$	$58^{\pm0.29\text{ aA}}$	57.0
	V	$31^{\pm0.39\text{ bcA}}$	$32^{\pm0.19\text{abB}}$	$34^{\pm0.41\text{ abA}}$	32.3	$54^{\pm0.54\text{ abA}}$	$55^{\pm0.62\text{ bA}}$	$56^{\pm0.21\text{ bA}}$	55.0
	VI	$28^{\pm0.63\text{ cB}}$	$31^{\pm0.39\text{ cA}}$	$31^{\pm0.72\text{ bA}}$	30.0	$52^{\pm0.42\text{ bB}}$	$53^{\pm0.51\text{ bcAB}}$	$54^{\pm0.44\text{ cB}}$	53.0
$\overline{X}$		31.5	33.3	34.0		53.3	54.2	54.3	
2023	I	$30^{\pm0.39\text{ abB}}$	$33^{\pm0.62\text{ aA}}$	$33^{\pm0.28\text{ abA}}$	32.0	$52^{\pm0.69\text{cA}}$	$53^{\pm0.19\text{dA}}$	$53^{\pm0.51\text{deA}}$	52.7
	II	$31^{\pm0.64\text{ abB}}$	$33^{\pm0.29\text{ aA}}$	$34^{\pm0.55\text{ aA}}$	32.7	$53^{\pm0.55\text{bcB}}$	$55^{\pm0.66\text{cA}}$	$54^{\pm0.43\text{dAB}}$	54.0
	III	$29^{\pm0.48\text{ bB}}$	$31^{\pm0.52\text{ bA}}$	$32^{\pm0.48\text{ bA}}$	30.7	$53^{\pm0.31\text{bcA}}$	$52^{\pm0.43\text{dA}}$	$52^{\pm0.62\text{eA}}$	52.3
	IV	$32^{\pm0.51\text{ aB}}$	$34^{\pm0.60\text{ aB}}$	$34^{\pm0.32\text{ aB}}$	33.3	$56^{\pm0.71\text{aB}}$	$57^{\pm0.52\text{bAB}}$	$58^{\pm0.55\text{bA}}$	57.0
	V	$30^{\pm0.30\text{ abB}}$	$33^{\pm0.68\text{ aB}}$	$33^{\pm0.39\text{ abB}}$	32.0	$54^{\pm0.41\text{bB}}$	$55^{\pm0.61\text{cAB}}$	$56^{\pm0.31\text{cB}}$	55.0
	VI	$31^{\pm0.72\text{ abB}}$	$34^{\pm0.36\text{ aB}}$	$33^{\pm0.44\text{ abA}}$	32.7	$58^{\pm0.33\text{aB}}$	$59^{\pm0.72\text{aAB}}$	$60^{\pm0.41\text{aB}}$	59.0
		30.5	33.0	33.2		54.3	55.2	55.5	

Tukey's multiple range test: p≤ 0.05, small letters a, b, c for the column, capital letters A, B, C for the row

Table 5. Correlation coefficients (r) between mean monthly air temperatures and seed quality of six locations at three different temperatures – *T. incarnatum*

Year	Mean monthly air temperatures	GS 15 C°	GS 20 C°	GS 25 C°	DS 15 C°	DS 20 C°	DS 25 C°
2021	March	-0.480 ns	-0.599 ns	-0.607 ns	0.652 ns	0.568 ns	0.720 ns
	April	-0.400 ns	-0.527 ns	-0.531 ns	0.726 ns	0.619 ns	0.737 ns
	May	0.269 ns	0.163 ns	0.092 ns	**0.965 ** **	**0.931 ** **	**0.893 ***
2022	March	0.134 ns	0.154 ns	-0.027 ns	0.755 ns	0.787 ns	0.628 ns
	April	0.521 ns	0.515 ns	0.359 ns	0.612 ns	0.699 ns	0.430 ns
	May	0.621 ns	0.619 ns	0.455 ns	**0.965 ** **	**0.975 *** **	**0.855 ***
2023	March	0.034 ns	0.162 ns	-0.003 ns	0.237 ns	0.333 ns	-0.111 ns
	April	0.711 ns	0.458 ns	0.548 ns	0.378 ns	0.367 ns	0.617 ns
	May	0.468 ns	0.534 ns	0.339 ns	**0.880 ***	**0.869 ***	**0.825 ***

Tukey's multiple range test, * p≤0.05, **p≤0.01, ***p≤0.001, ns not signifcant (p≥0.05)

Table 6. Correlation coefficients (r) between monthly amount of rainfall (mm) and seed quality of six locations at three different temperatures – *T. incarnatum*

Year	Monthly amount of rainfall mm	GS 15 C°	GS 20 C°	GS 25 C°	DS 15 C°	DS 20 C°	DS 25 C°
2021	March	-0.770 ns	-0.652 ns	-0.648 ns	-0.489 ns	-0.417 ns	-0.415 ns
	April	-0.383 ns	-0.340 ns	-0.221 ns	-0.234 ns	-0.183 ns	-0.127 ns
	May	**-0.899***	**-0.819***	**-0.860***	-0.352 ns	-0.176 ns	0.021 ns
2022	March	-0.051 ns	-0.094 ns	0.043 ns	-0.367 ns	-0.630 ns	-0.470 ns
	April	-0.571 ns	-0.605 ns	-0.401 ns	-0.367 ns	-0.630 ns	-0.470 ns
	May	**-0.853***	**-0.883***	**-0.918****	0.526 ns	0.698 ns	0.778 ns
2023	March	0.119 ns	-0.059 ns	-0.233 ns	0.417 ns	0.122 ns	0.245 ns
	April	0.341 ns	-0.084 ns	0.400 ns	0.012 ns	-0.005 ns	-0.014 ns
	May	**-0.823***	**-0.873***	**-0.815***	-0.541 ns	-0.800 ns	-0.739 ns

Tukey's multiple range test, * p≤0.05, **p≤0.01, ***p≤0.001, ns not signifcant (p≥0.05)

For seeds collected between 2021 and 2023, dormancy and germination were tested at 15 °C, 20 °C, and 25 °C. Correlation analysis between mean monthly temperatures and seed germination at these test temperatures revealed no significant relationships ($p \geq 0.05$) for any of the three months across the six locations (Table 5). However, seed dormancy assessed at the same temperatures showed a significant positive correlation with May temperatures across all locations and years, (r=$p \leq 0.05$ to $p \leq 0.001$. In contrast, no significant correlation was observed between dormancy and mean temperatures in March or April ($p \geq 0.05$) (Table 5).

Table 7. Stem and root growth (cm) of seeds in six locations under different meteorological conditions (2021–2023)

Year	Locations	Stem cm				Root cm			
		15 C°	20 C°	25 C°	$\overline{X}$	15 C°	20 C°	25 C°	$\overline{X}$
2021	I	1.995 bB	2.167 bA	2.195bA	2.119	1.312 baB	1.365 bA	1.378 bA	1.352
	II	2.119 aB	2.195 abA	2.199bA	2.171	1.469 abB	1.489 abA	1.499 abA	1.486
	III	2.045 abB	2.189 abA	2.231abA	2.155	1.357 abB	1.371 bA	1.382 bA	1.370
	IV	2.121 abB	2.269 aA	2.286 aA	2.225	1.403 abA	1.452 abA	1.468 abA	1.441
	V	2.192 aB	2.239 abA	2.256a bA	2.229	1.504 aB	1.558 aA	1.562 aA	1.541
	VI	2.028 abB	2.198 abA	2.218abA	2.148	1.338 bB	1.368 bA	1.372 abA	1.359
$\overline{X}$		2,083	2.210	2.231		1.397	1.434	1.444	
2022	I	1.812 bB	1.993 bA	2.005 bA	1.937	1.267 abB	1.298 abB	1.299 abB	1.288
	II	2.037 aB	2.189 abA	2.297 abA	2.174	1.305 aB	1.351 aA	1.362 aA	1.339
	III	1.969 abB	2.195 abA	2.221 abA	2.129	1.304 aB	1.349 aA	1.354 aA	1.336
	IV	2.004 aB	2.362 aA	2.484 aA	2.283	1.294 abB	1.339 abA	1.342 abA	1.325
	V	1.903 abB	2.165 abA	1.192 b A	1.753	1.217 bB	1.263 abA	1.264 abA	1.248
	VI	1.838 abB	1.997 bA	2.011abA	1.949	1.209 bB	1.245 bA	1.248 bA	1.234
$\overline{X}$		1,927	2.150	2.035		1.266	1.308	1.312	
2023	I	2.074abB	2.275abA	2.312abA	2.220	1.465aB	1.489aA	1.496aA	1.483
	II	2.019abB	2.246abA	2.251abA	2.172	1.448abB	1.472aA	1.478abA	1.466
	III	2.006bB	2.256abA	2.272abA	2.178	1.455abB	1.469abA	1.470abA	1.465
	IV	2.119aB	2.289abA	2.342aA	2.250	1.451abB	1.473abA	1.478abA	1.467
	V	1.999bB	2.214bA	2.224bA	2.146	1.405bB	1.431bA	1.433bA	1.423
	VI	2.102bB	2.298aA	2.311abA	2.237	1.396bB	1.461abA	1.468abA	1.442
$\overline{X}$		2.053	2.263	2.285		1.437	1.466	1.471	

Tukey's multiple range test: p≤ 0.05, small letters a, b, c for the column, capital letters A, B, C for the row

Over the three-year period, no significant correlation was observed between precipitation in March and April and seed germination at 15 °C, 20 °C, or 25 °C. In contrast, May precipitation showed a significant negative correlation with seed germination at all three temperatures ($p \leq 0.05$ to $p \leq 0.01$). Additionally, no significant relationship was found between precipitation in March, April, or May and seed dormancy at any of the tested temperatures (Table 6).

Table 8. Correlation coefficients (r) between mean temperatures and seedling growth of six locations at three different temperatures

Year	Mean monthly air temperatures	Stem cm 15 ºC	Stem cm 20 ºC	Stem cm 25 ºC	Root cm 15 ºC	Root cm 20 ºC	Root cm 25 ºC
2021	March	-0.421 ns	-0.325	-0.069	-0.503	-0.392	-0.402
	April	0.090 ns	0.117	0.282	-0.037	0.064	0.031
	May	-0.594 ns	-0.694	-0.531	-0.546	-0.481	-0.507
2022	March	-0.367	0.105	-0.100	-0.179	-0.195	-0.248
	April	-0.556	-0.417	-0.681	-0.375	-0.382	-0.408
	May	0.403	0.442	0.278	-0.117	-0.061	-0.039
2023	March	0.465	0.517	0.622	0.135	0.137	0.088
	April	-0.123	-0.222	-0.071	0.244	-0.147	-0.194
	May	-0.355	-0.037	-0.023	0.517	0.341	0.259

Tukey's multiple range test, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns not signifcant ($p \geq 0.05$)

Table 9. Correlation coefficients (r) between monthly amount of rainfall (mm) and seedling growth of six locations at three different temperatures

Year	Monthly amount of rainfall mm	Stem cm 15	Stem cm 20	Stem cm 25	Root cm 15	Root cm 20	Root cm 25
2021	March	-0.549	-0.238	-0.361	-0.579	-0.499	-0.506
	April	-0.489	-0.174	-0.290	-0.545	-0.461	-0.464
	May	0.007	0.219	0.171	-0.119	-0.117	-0.129
2022	March	-0.390	-0.190	0.278	-0.376	-0.428	-0.434
	April	-0.226	-0.191	0.029	-0.595	-0.586	-0.564

	May	0.009	0.070	0.210	-0.396	-0.373	-0.353
2023	March	-0.463	-0.565	-0.720	-0.784	-0.757	-0.711
	April	0.071	0.193	-0.140	-0.695	-0.235	-0.170
	May	0.264	0.326	0.029	-0.586	-0.095	-0.018

Tukey's multiple range test, * p≤0.05, **p≤0.01, ***p≤0.001, ns not signifcant (p≥0.05)

Shoot and root growth. For all seeds locations and all years, shoot and root growth were significantly higher (p≤0.05) following germination at 20 ºC and 25 ºC compared to 15 ºC, while no significant difference was observed between growth after germination at 20 ºC and 25 ºC (p≥0.05) (Table 7).

No statistically significant difference (p≥0.05) was found between the relationship (r) of root and shoot growth and the monthly temperatures in March, April, and May over the three-year period (Table 8). Similarly, no significant correlation (p≥0.05) was found between root and shoot growth and monthly precipitation in March, April, and May from 2021 to 2023 (Table 9).

4. DISCUSSION

Many analyses indicate that we are experiencing a period of increased and variable average temperatures and irregular precipitation, often with extreme events, which inevitably impact agricultural production (Alvar-Beltrán & Franc-eschini, 2024; Messeri et al., 2024).

Our research confirms this, showing high variability in both mean monthly temperatures and precipitation during March, April, and May across all years (2021, 2022, 2023) and six locations in Eastern Serbia (Table 1).

According to Willis et al., (2014), varying percentages of seed dormancy occur depending on climatic conditions and plant species, driven by complex physiological and biochemical mechanisms.

Over centuries, humans have domesticated plants and improved many traits—such as reduced seed dormancy and increased germination—to better align crop establishment with agroecological conditions (Purugganan, 2019). However, in species where breeding has been less intensive, dormancy levels remain similar to those of their wild relatives (Wen et al., 2024).

In legumes, water-impermeable seed coats prevent germination even under favorable agroecological conditions (Rezaei-Manesh et al., 2023; Fu et al., 2024).

According to Baskin and Baskin (2004), seed dormancy can be physiological, morphological, morphophysiological, physical, or a combination thereof. However, in legumes, physical dormancy is most common due to impermeable seed coats. Penfield & MacGregor (2017) note that seed coat structure is highly variable and strongly influenced by the environment, affecting seed dormancy depending on the species. The mechanisms regulating these processes primarily maintain a dynamic balance between abscisic acid and gibberellins (Nambara et al., 2010).

Baill & Gomez Roldan (2023) highlight that the seed-filling stage is critical, involving synthesis, mobilization, and transport of proteins, carbohydrates, lipids, and other compounds. This process is highly sensitive to environmental changes, which can cause both qualitative and quantitative shifts in seed traits.

In physically dormant seeds, such as those of many legumes, the seed coat prevents water uptake, thereby inhibiting embryo development (Bolingue et al., 2010). This physical dormancy, characteristic of Fabaceae (Leguminosae) family members—especially forage crops from the *Trifolium* (Štrbanović et al., 2025; Rezaei-Manesh et al., 2023; Can et al., 2009) and *Medicago* (Wu et al., 2023; Xie et al., 2023; Renziet al., 2020; Galussi et al., 2013) genera—is well documented.

According to Pawłowski at al. (2020) the dormancy cycling phenomenon has been widely studied, but the molecular mechanism responsible remains largely unknown. Seed dormancy breaking and germination is a multifaceted process, associated with changes in the gene expression, protein synthesis, and physiology, but also with organelle functioning (Née, et al., 2017).

Our findings for *T. incarnatum* suggest that higher temperatures increase the synthesis of water-impermeable compounds in the seed coat, directly raising the percentage of dormant seeds. This is supported by positive correlation coefficients between mean monthly temperatures and seed dormancy ($p \leq 0.05$ to $p \leq 0.001$) (Table 5).

In contrast, higher May precipitation negatively affects seed germination, as indicated by significant negative correlations between rainfall amounts and germination percentages ($p \leq 0.05$ to $p \leq 0.01$) (Table 6).

According to Bailly & Gomez Roldan (2023), temperature and water availability directly influence seed development and germination. Therefore, examining seed quality in response to climate change is a step toward recommending new cultivars and agronomic practices to address future challenges.

5. CONCLUSION

These findings suggest that early spring precipitation (March and April) does not significantly influence the germination or dormancy of crimson clover seeds under given temperature conditions, indicating a degree of resilience in seed physiological development during this period. In contrast, during the later stages of development (seed filling and ripening in May), increased rainfall has a significant negative effect on seed germination. During this same period, higher temperatures lead to an increased proportion of dormant seeds.

From an agricultural perspective, these results underscore the importance of monitoring and managing precipitation patterns during the late reproductive phase of crimson clover. Furthermore, site selection for seed production should consider agroclimatic conditions, especially during the seed-filling and maturation stages (May), to minimize the risk of impact of precipitation on seed quality. If climate change leads to higher May temperatures, it is realistic to expect increased seed dormancy, highlighting the need to implement new pre-sowing technologies to improve germination and early seedling growth.

Further studies are warranted to identify and optimize seed treatment methods that effectively break dormancy and improve germination performance in crimson clover, thereby supporting more consistent establishment in field conditions.

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