



## Utilizing of Plant-Smoke Solution to Alleviate Drought Sensitivity on Forage Peas

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### HIGHLIGHTS

- Drought stress is a meteorological event that most threatens agricultural production and food security in the future.
- Therefore, urgent measures need to be taken to preserve the quality and yield of existing genotypes.
- In this context, smoke solutions in which both natural and agricultural wastes are recycled can be used.
- Because, the results of this study determined that priming with smoke solution prevented the effects of drought on forage pea.

### Abstract

The study was conducted to determine the sensitivity of forage pea to drought stress intensities and the contribution of smoke solutions in preventing this sensitivity. For the smoke solution, dried poppy harvest residues were prepared with a special mechanism. Within this scope, two different concentrations (1 and 10%) of poppy smoke solution were utilized in priming of two distinct forage pea varieties (Gap pembesi and Özkaynak). After priming, the seeds were sowing in pots and it was exposed to moderate and severe drought conditions after 21 days. Effects of smoke solutions and drought stress in varieties were assessed by physically (shoot length) and chemical parameters (crude protein, ADF, NDF, Ca, Mg, P and K). Both forage pea varieties have been observed that crude protein and mineral substance (except Ca) contents decrease significantly under drought stress, especially in severe drought. This loss was increased again by eliminating both doses (especially 10%) of smoke solutions. In fact, under normal growing conditions (control), 10% smoke solution application had the highest crude protein in both varieties. However, the effect of drought and the defense mechanism of smoke solution created differences in forage pea varieties based on parameters. In general, the Özkaynak was more resistant to drought and the response to smoke solution was stronger. In conclusion, by incorporating the poppy-smoke solution into the drought stress mitigation strategy, this approach not only curbed environmental losses but also mitigated the impacts of drought stress.

**Keywords:** Smoke solution; drought; forage quality; priming.

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## 1. Introduction

The formation of growth and quality in plants is primarily an extremely complex process determined by both genetics and the environment. While there may not be genetic variability, the environment, especially due to climate change, is constantly undergoing changes. In recent years, with global climate change, the consequences of commonly observed extreme meteorological events have been gaining increasing attention worldwide. Drought, along with other abiotic stresses, is a meteorological event that has shown a growing trend in terms of its duration, frequency, and spatial scope and significantly affects agricultural production.

By the year 2050, it is estimated that the global population will exceed 9.7 billion, with more than 65% of individuals relying solely on agriculture for their livelihoods. In developing countries like Turkey, this ratio is predicted to reach as high as 90% (Castañeda et al. 2016). In contrast, 22.5% of Turkey is experiencing high desertification, while 50.9% is showing a moderate tendency towards desertification. This situation reflects the severity of the threats that will emerge in the future. Therefore, making a paradigm shift towards sustainable agriculture and finding solutions to water scarcity and its impact on food security are of vital importance (Khaleghi et al. 2019). The need to develop methods that can increase a plant's resistance to drought stress, improve crop growth, and contribute to environmentally friendly and sustainable agriculture has become essential.

Drought severely limits the morpho-physiological functions of plants, having a negative impact on plant growth, productivity, reproduction, and survival. It is known that the increase in reactive oxygen radicals within plant cells, induced by abiotic stresses, is at the core of these negative impacts (Zandalinas et al. 2020). Plants adapt or respond to changing environmental conditions through various morphological, physiological, anatomical, and biochemical changes. Mechanisms and responses specific to drought stress can vary widely among different plant species and even varieties. Therefore, in our study, two different varieties of forage pea were examined in drought conditions.

The protein content of forage peas, which can reach up to 40%, indicates that it is a quality forage crop. It plays a significant role in improving animal nutrition, and in the future, there will be an increased need for high-quality and high-yielding varieties due to drought stress. Yield and quality are directly influenced by environmental factors and their interactions. Understanding the expression of these effects at the phenotypic and biochemical levels and predicting the segregation of field-assessed products are of great importance. In this context, it is necessary to first evaluate the responses of forage peas, which are sensitive to drought, to drought severity and to apply techniques that will mitigate or eliminate these responses.

Various approaches have been employed to mitigate drought stress, as documented in studies by Saha et al. (2020) and Zhang et al. (2020). Nevertheless, an emerging and relatively underappreciated method within the realm of agricultural mitigation strategy involves the use of plant-derived smoke solutions. This innovative approach contributes to environmentally sustainable solutions aimed at ensuring global food security. These smoke solutions, derived from burning agricultural waste and preserving the resulting smoke in water, can be applied directly to seeds through priming or to the soil. Several researchers have demonstrated the significant positive impact of plant-derived smoke solutions on germination and seedling growth, as evidenced in studies by Jefferson et al. (2008), Dixon et al. (2009), and Doğrusöz (2022). Furthermore, it has been established that smoke solutions enhance plant tolerance to abiotic stresses, as highlighted in studies by Li et al. (2017), Shah et al. (2021), Shah et al. (2020), and Khan et al. (2017). However, it should be noted that the efficacy of these smoke solutions varies depending on factors such as the type and quantity of plant material burned, the solution concentration, and the specific plant genotype employed (Doğrusöz et al. 2022).

This study focused on assessing the sensitivity of two distinct forage pea varieties to moderate and severe drought stress using chemical parameters. Additionally, two different concentrations of poppy smoke solution were utilized in priming to alleviate drought sensitivity. By incorporating the poppy-smoke solution into the drought stress mitigation strategy, this approach not only curbed environmental losses but also mitigated the impacts of drought stress.

## 2. Materials and Methods

The varieties 'Gap Pembesi and Özkaynak' of forage pea (*Pisum sativum* spp. *arvense* L.) were used by plant material. For the smoke solution, dried poppy harvest residues were used. The separate trials for both varieties were performed under fully controlled climate room (light, temperature and humidity) at the Agriculture Faculty of Yozgat Bozok University.

### *Priming Application with Smoke Solutions*

Smoke solutions were generated from the poppy residues. The 1 kg of poppy straw was subjected to controlled combustion using a specialized system, following the methodology detailed in studies by Ghebrehiwot et al. (2009) and Basaran et al. (2019). The resulting smoke was then entrapped by passing it through 4 liters of distilled water. The initial smoke solution was subsequently diluted to achieve concentrations of 1% and 10% using additional distilled water. Forage pea seeds were soaked for 18 hours at a temperature of 22°C in these smoke solutions, while distilled water served as the control group.

### *Forage pea Growth Conditions, Drought Stress Treatments*

The study encompassed three distinct treatment groups: severe and moderate drought, and normal conditions (irrigation; control). Certainly, the drought stress conditions were created by subjecting the plants to reduced water availability. Specifically, severe drought stress was simulated by withholding water for the final eight days of the experiment, while moderate drought stress was imposed by withholding water for the last four days. This manipulation aimed to replicate varying degrees of water scarcity to study the plants' responses under different levels of drought stress. That is, drought stress was achieved by completely cutting off water from the seedlings. The primed seeds of these plants were sown in 8-liter pots filled with a consistent mixture of peat and soil. Employing randomized plots trial design, the experiment featured three replications, each comprising 10 plants. The trials were conducted separately. Both varieties of forage pea were meticulously cultivated under controlled conditions, maintaining a temperature of  $25 \pm 2^\circ\text{C}$ . Subsequently, all plants were harvested 28 days after the experiment's initiation.

### *The Parameters Examined in Forage Pea Seedlings*

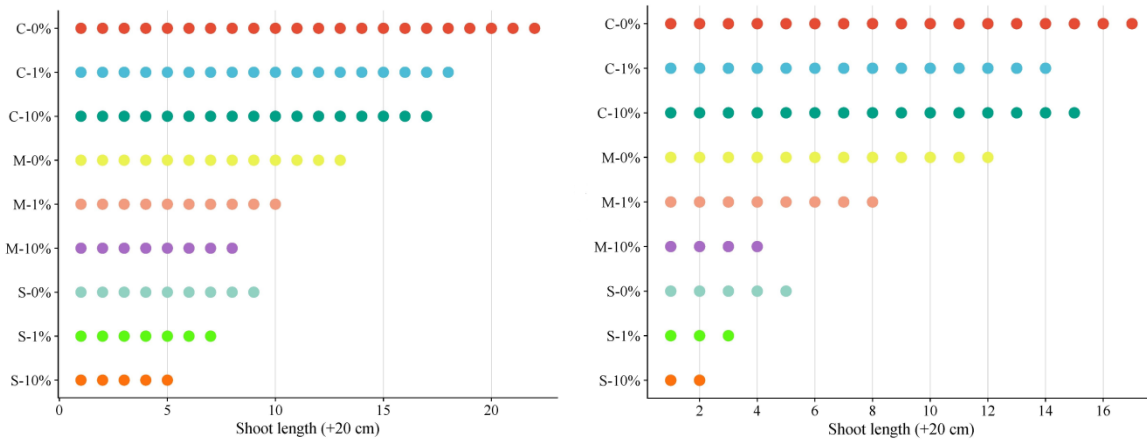
Twenty-eight days later, all plants in the pots were removed and shoot length (cm) was determined. The samples were dried in a drying cabinet at 65 °C for 48 hours and ground to a diameter of 1 mm. Dried samples were ground and passed through a 1 mm sieve. The ground samples were processed in NIRS (Foss 6500) device (near infrared reflectance spectroscopy; Silver Spring, MD, USA) with the IC0904FE program to determine crude protein (PRT;%), ADF (acid detergent fiber,%), NDF (neutral detergent fiber;%). and mineral matter (Ca, P, K and Mg;% ) were analyzed.

### *Statistical analyses*

Statistical analyses of data were conducted with SPSS software, version 20.0 Duncan's test was performed to assess differences between averages at a significance level of  $p \leq 0.05$ . All parameters' data were further subjected to correlation analysis and a principal component analysis (PCA), with separate for each variety. The heat map, PCA and ballon plot graphics were created with SRplot (Tang et. al. 2023), separately for each variety

## 3. Results

Moderate and severe stresses of drought significantly influenced forage pea plant growth and chemical contents. However, the smoke solution applied alleviated the negatives of these effects. In both varieties, the longest shoot length was determined in 0 dose of the control treatment. The expected decrease in shoot length was observed with drought stress. However, smoke solutions significantly reduced shoot length in both varieties, both in normal irrigation and drought stress treatments. Therefore, smoke has a negative effect on the solution.



**Figure 1.** Changes in shoot length of smoke solution applied in forage pea under drought stress (*Gap pembedesi*: left graphic, *Özkaynak*: right graphic)

In both varieties, chemical contents were significantly ( $p < 0.01$ ) affected by priming with smoke solutions under applied drought stress (Table 1 and 2). The highest protein content in both varieties was obtained under normal irrigation conditions and 10% solution application. Additionally, Crude protein content increased in moderate drought as a defense mechanism against stress but decreased in severe drought. However, it was observed that the protein content increased in parallel with the increase in smoke solution in drought applications as with normal irrigation.

**Table 1.** Chemical composition (%) of smoke solution applied in *Gap Pembedesi* under drought stress

Treatments	PRT	ADF	NDF	Ca	Mg	P	K
C-0%	31.98 bc	14.30 bc	27.69 ab	1.72 cd	0.54 c	0.39 cd	2.38 c
C-1%	32.16 bc	14.76 b	27.33 ab	1.97 bc	0.55 c	0.39 cd	2.44 c
C-10%	35.29 a	13.86 c	30.13 a	2.00 b	0.58 b	0.45 b	2.90 b
M-0%	32.17 bc	14.65 b	27.61 ab	1.63 d	0.48 d	0.38 cd	1.91 d
M-1%	32.85 b	10.20 d	20.70 c	1.81 c	0.50 cd	0.43 bc	2.93 b
M-10%	32.46 b	14.12 bc	25.72 b	2.27 ab	0.63 ab	0.48 a	3.37 a
S-0%	31.00 c	15.34 a	24.13 b	1.99 bc	0.52 cd	0.37 d	1.87 d
S-1%	31.97 bc	13.08 cd	21.67 c	2.16 b	0.59 b	0.40 c	2.33 cd
S-10%	32.70 b	9.56 d	17.57 d	2.41 a	0.67 a	0.41 c	2.69 bc

Different letters significant differences at  $p \leq 0.05$  within one parameter. PRT; crude protein, C; control, M; moderate drought, S; severe drought.

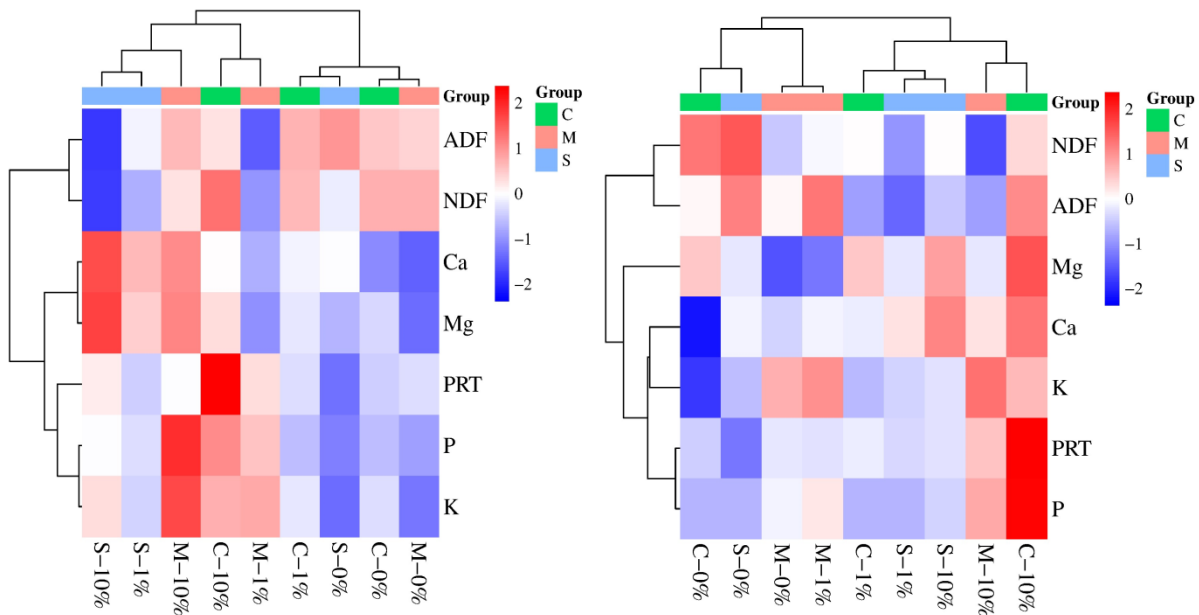
An increase in the ADF of both varieties was determined under severe drought. However, in *Gap pembedesi*, smoke solution application caused a decrease in ADF in all three cultivate conditions. In *Özkaynak*, while ADF increased in 10% dose under normal conditions, it increased in 1% dose under moderate drought. In *Gap pembedesi*, NDF content was higher under normal irrigation condition than in other treatments. Drought stress and smoke solution applications generally reduced NDF content. In *Özkaynak*, NDF decreased in moderate drought and increased in severe drought. These values generally decreased in smoke solution applications. In smoke solution applications, these values decreased with 1% solution but increased again with 10% solution, in drought conditions (Table 1 and 2).

**Table 2.** Chemical composition (%) of smoke solution applied in Özkaynak under drought stress

Treatments	PRT	ADF	NDF	Ca	Mg	P	K
C-0%	29.29 c	21.58 b	37.56 ab	1.01 e	0.37 bc	0.46 c	3.48 d
C-1%	30.02 bc	19.60 cd	34.89 c	1.29 cd	0.37 bc	0.46 c	3.86 c
C-10%	35.94 a	23.59 ab	35.54 bc	1.49 a	0.40 a	0.56 a	4.27 b
M-0%	29.91 bc	21.53 b	33.79 cd	1.26 cd	0.31 e	0.48 bc	4.30 b
M-1%	29.81 bc	24.02 a	31.27 d	1.30 c	0.32 d	0.49 bc	4.40 ab
M-10%	31.61 b	19.53 cd	34.62 c	1.35 b	0.35 cd	0.51 b	4.49 a
S-0%	27.45 d	23.84 a	38.08 a	1.30 c	0.35 cd	0.46 c	3.88 c
S-1%	29.52 c	18.48 d	32.71 cd	1.35 b	0.35 cd	0.46 c	3.94 bc
S-10%	29.77 bc	20.32 c	34.89 c	1.47 a	0.38 b	0.47 bc	3.98 bc

Different letters significant differences at  $p \leq 0.05$  within one parameter. PRT; crude protein, C; control, M; moderate drought, S; severe drought.

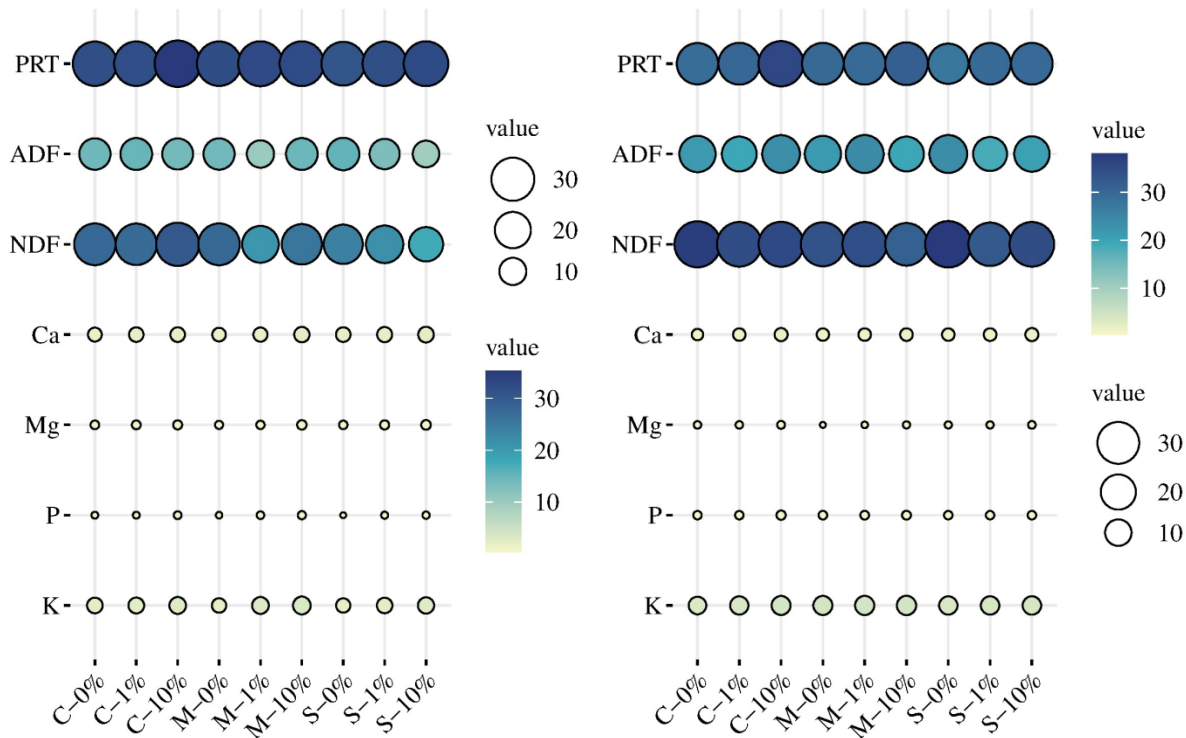
In *Gap pembesi*, mineral substance contents (Ca, Mg, P and K) decreased in parallel with the stress severity under drought conditions. As a result of priming with smoke solutions, it was determined that a 10% dose increased the mineral substance content in both moderate and severe drought. Moreover, M-10% application had the maximum potassium and phosphorus content, while M-10% and S-10% applications had the maximum calcium and magnesium content (Table 1). In *Özkaynak*, mineral matter contents decreased under drought stress. However, these decreases were prevented by smoke solution doses. Additionally, calcium, magnesium and phosphorus reached their maximum in C-10% application. Potassium content had the highest value in the M-5% and M-10% treatments (Table 2).



**Figure 2.** Representation with a heat map of the relationship between responses of smoke solution applied in forage pea under drought stress (*Gap pembesi*: left graphic, *Özkaynak*: right graphic), (The heat map was created with SRplot)

A heat map serves as a means to visually represent and analyze intricate data sets, commonly applied in various fields of data analysis and statistics. In this study, separate heat maps were generated for each genotype to illustrate the relationships between features and applications, as well as to depict the distribution of applications and features within the context of these relationships (Figure 2). The color patterns on the map distinctly highlight the influence of the growing environment and the impact of drought concerning the examined characteristics in pea genotypes. The influence of smoke solutions is evident in both varieties; however, this graph clearly demonstrates that the *Özkaynak* variety exhibits a notably positive response to

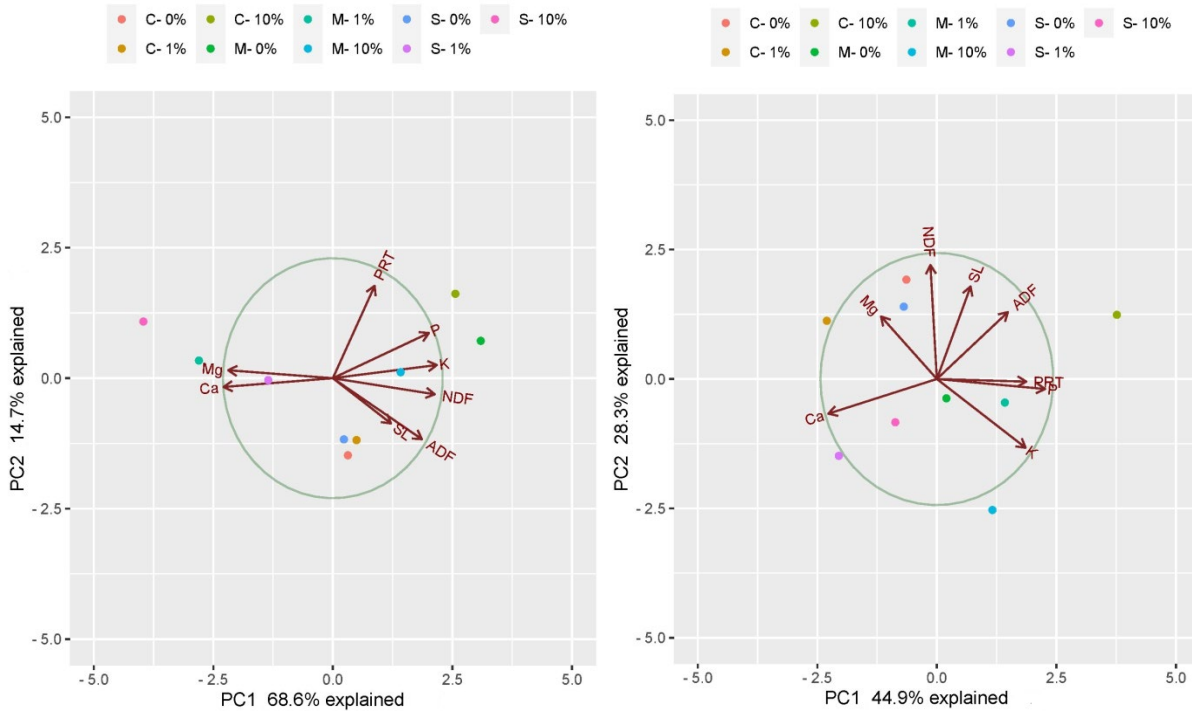
the 10% smoke solution. Balloon plot graphics, which classify the effects of features on treatments according to circle size and color tones, are also designed for each type (Figure 3). It is clearly seen that the PRT in both varieties increases in parallel with the smoke solution ratios and is high in the C-10% application. Circle sizes vary depending on the values of the features, and the highest effect was on the protein content in Gap pembesi, while it was on NDF in Özkaynak.



**Figure 3.** Representation with the balloon plot of the responses of smoke solution applied to forage pea under water deficiency conditions. The mean values of the replicates are shown (*Gap pembesi*: left graphic, *Özkaynak*: right graphic), (The balloon plot was created with SRplot).

In this research, principal component analysis (PCA) was utilized to delve into the intricacies of eight variables and unveil their fundamental characteristics. Additionally, PCA was employed to observe the collective impact of all transactions on graphs for each genotype, using two components. The PCA biplots provided a comprehensive insight into the biochemical responses of forage pea to water deficiency and smoke solutions. Separate PCA biplots were generated for both the population and variety of forage pea. For *Gap pembesi*, the biplot graphs elucidated 69.63% of the cumulative variance, with PC1 and PC2 contributing 68.6% and 14.7% respectively. Conversely, *Özkaynak* exhibited principal components PC1 and PC2, representing 44.9% and 28.3% of the cumulative variance, respectively (Figure 4). Vector contributions in the biplot indicated that the first component was heavily loaded by almost all traits, with a similar trend for the second component, except for SL. Analysis revealed four main groups in both biplots, as evidenced by the heat map. In both varieties, grouping was influenced by drought stress and smoke solutions, although the specifics varied primarily based on the dose of smoke solutions. Priming, particularly with smoke solution, led to a reduction in these fundamental distinctions, causing different treatments to converge into the same groups. Notably, in *Özkaynak*, treatments C-10 and MD-10% were placed in the same group. In *Gap pembesi*, the M-10% treatment occupied positive quadrants of both F1 and F2, whereas in *Özkaynak*, C-10% treatments did. Additionally, it was apparent that the response mechanism to drought stress in *Gap pembesi* was linked with an increase in PRT, K, and P, while in *Özkaynak*, it was associated with an increase in PRT, SL, and ADF.

These findings underscored the significant efficacy of poppy smoke solution in enhancing drought stress tolerance (Figure 2, 3 and 4).



**Figure 4.** Principal component analysis of the responses of smoke solution applied to forage pea under water deficiency conditions. The mean values of the replicates are shown (*Gap pembesi*: left graphic, *Özkaynak*: right graphic).

#### 4. Discussion

The study examined the physical and chemical reactions to water deficiency in various forage pea varieties, aligning with prior research (Gökmen and Ceyhan 2015; Ceyhan et al. 2012; Reinhardt et al. 2015; Yousofi et al. 2016; Tekin and Ceyhan 2022). The findings suggest that forage pea varieties exhibit sensitivity to unfavorable ion ratios, and osmotic stress, such as drought, impacts cell division. The parameters examined in the study were evaluated as defense mechanisms against drought stress and are very important in terms of animal nutrition. The balance between protein with minerals and fiber components with ADF and NDF values, is crucial for assessing the nutritional quality of forage crops. Livestock performance, including growth rates, milk production, and overall health, can be significantly influenced by the crude protein, minerals and fiber content in their diet. Therefore, understanding these parameters helps farmers and nutritionists make informed decisions about the suitability of forage crops for specific livestock needs. It may involve adjusting feeding practices, supplementing with additional nutrients, or seeking alternative forage sources during periods of drought to maintain the health and productivity of livestock. Instead, it is much more logical and profitable to preserve the existing potential of quality forages, such as forage peas, with practices that will prevent quality loss under drought conditions.

In both drought stress levels were generally negative effects on the nutritional composition of plants, including changes in crude protein, mineral content, and fiber fractions such as ADF and NDF. In moderate drought, there was a partial increase in crude protein concentration as the plant tries to concentrate nutrients in response to water scarcity. However, in other instance, drought stress increase was reducing protein synthesis and result in lower crude protein content. Drought stress in both forage pea was typically leads to



an increase in fiber content, including both ADF and NDF. As the plant experiences water scarcity, it may allocate resources to the production of structural components like cellulose and lignin, resulting in higher fiber concentrations. However, since the results obtained were within the range required for quality hay (NRC, 2001; 25-35% ADF and 40-50%), this increase was considered positive in the study. Water scarcity can limit the uptake of essential minerals (Ca, Mg, from the soil, affecting the overall nutrient profile of the plant. Mineral contents had variable effects on drought stress levels, decrease in especially severe drought, but Ca content increased in both varieties. Additionally, all of the mineral substance contents obtained in the study were found to be above the values (Kidambi et al. 1993; min. 0.8% K, 0.21% P, 0.3% Ca and 0.1% Mg) that should be in quality forage. These similar effects have been determined by many researchers in plants exposed to drought stress (Pei et al. 2010; Chen et al. 2011). The specific responses were vary among plant species and depend on the severity and duration of drought stress (Hu and Schmidhalter 2005). Additionally, some plants may exhibit adaptive responses to drought, such as altering their physiological processes to better withstand water scarcity (Seleiman et al. 20219). However, it is clearly seen in this study that decreased the PRT, Mg, K and P contents with drought stress in forage pea was increased again with poppy-derived smoke solutions. However, the effect of drought and the defense mechanism of smoke solution created differences in forage pea genotypes. In general, the Özkaynak was more resistant to drought and the response to smoke solution was stronger.

Plant-derived smoke solutions, known for their significant impact on germination, root growth and seedling development (Dogrusoz 2022), effectively mitigated the forage pea's response to drought stress (Li et al. 2017). Recent research has identified that the protective effect against drought is due to in strigolactones and karrikins butenolide molecules, present in plant-derived smoke solutions. These components have been shown to positively influence various processes related to plant drought responses, including the regulation of chemical composition (Li et al. 2017; Yang et al. 2020; Zheng et al. 2020). In the context of drought, the application of smoke solutions might help plants overcome stress and improve their resilience. However, the specific effects can vary depending on the plant species, doses and environmental conditions.

## 5. Conclusions

These results showed that in forage pea, poppy smoke solutions helped them cope with the adverse effects of drought by activating specific physiological and chemical responses. However, the defense mechanism against drought stress of the Özkaynak variety was stronger. It's important to note that the use of smoke solutions in agriculture and ecosystem restoration is an evolving field, and more research is needed to fully understand the mechanisms involved and the potential benefits across different plant species and environments. Therefore, plant-based applications of smoke solutions in drought stress need to be increased. Also, we think that smoke solutions should be evaluated under other biotic and abiotic stress conditions as well as drought stress. Additionally, the practical application of these solutions in agriculture may vary, and considerations such as concentration, timing, and compatibility with other agricultural practices need to be addressed.

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