

IMPACT OF DROUGHT STRESS ON TOTAL PHENOLS, BUTANOL-SOLUBLE POLYPHENOLS, AND VANILLIN IN THREE FABA BEAN (*VICIA FABA* L.) CULTIVARS

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Abstract: The phenolic makeup of legumes plays a key role in their nutritional quality, antioxidant capacity, and how they respond to environmental stress. This study investigated how drought stress influences total phenols (TP), butanol-soluble polyphenols, and vanillin-reactive compounds in three faba bean (*Vicia faba* L.) cultivars: Reinablanca, Aquadulce L., and Alfred. Plants experienced both traditional (T) and selected (S) post-harvest treatments to examine how genotype and drought stress affect phenolic metabolism. TP levels ranged from 46.53 mg/g DM in Reinablanca T to 92.68 mg/g DM in Alfred S, with Alfred consistently showing the highest levels under drought conditions. Butanol-soluble polyphenols followed a similar trend, reaching 109.35.35 mg/g DM in Alfred S, which indicates a strong link between TP and polymerized phenolics. In contrast, vanillin content dropped under the selected treatment across all cultivars, with Reinablanca T having the highest level at 41.40 mg/g DM. This suggests a metabolic trade-off where stress-driven phenolic production may limit precursors for flavor-related compounds. The different cultivars responded uniquely: Alfred showed the highest potential for bioactive properties, Reinablanca maintained higher vanillin levels under traditional treatments, and Aquadulce L. exhibited intermediate traits but had higher butanol-soluble phenolics during drought stress. These findings show that drought stress boosts antioxidant phenolics while decreasing flavor-related vanillin, reflecting an adaptive shift in metabolism. Overall, Alfred stands out as a promising cultivar for functional foods or nutraceuticals owing to its strong antioxidant properties, while Reinablanca might be better suited for products focused on sensory qualities. This research offers valuable insights into how stress affects phenolic metabolism in legumes and guides strategies for selecting cultivars and managing post-harvest treatments to improve both nutritional and sensory qualities.

Keywords: *Vicia faba*, drought stress, total phenols, butanol-soluble polyphenols, vanillin, cultivar variation, antioxidant potential

1. Introduction

Leguminous crops, such as *Vicia faba* L. (faba bean), serve as an important source of plant proteins, vitamins, and bioactive compounds for both human and animal nutrition (Crépon *et al.*, 2010; Torres *et al.*, 2019). Besides their agronomic importance, faba beans are rich in phenolic compounds, including flavonoids, tannins, and phenolic acids, which play vital roles in plant defense, stress tolerance, and human health due to their antioxidant properties (Duenas *et al.*, 2006; Díaz *et al.*, 2017).

Abiotic stresses, especially drought, are major limiting factors impacting plant growth and crop yield worldwide. Drought causes oxidative stress by producing excess reactive oxygen species (ROS), leading to cellular damage and activating

defense responses, such as phenolic biosynthesis (Sharma *et al.*, 2012; Hasanuzzaman *et al.*, 2020). These secondary metabolites serve as powerful antioxidants, neutralizing free radicals and stabilizing cell membranes (Bulgari *et al.*, 2019). Consequently, measuring total phenolics (TP), condensed tannins (CTs), and vanillin-reactive compounds offers important insights into how plants physiologically and biochemically respond to water scarcity (Vargas-Ortiz *et al.*, 2021).

In *Vicia faba*, phenolic accumulation is associated with stress adaptation, particularly under drought and high temperatures (Ali *et al.*, 2018). The synthesis and buildup of specific compounds, such as butanol-soluble phenolics and vanillin-reactive compounds, are considered indicators of oxidative stress and

phenylpropanoid metabolism (Mierziak *et al.*, 2014). Previous research has demonstrated that environmental conditions, genotypic differences, and developmental stages significantly affect phenolic content in legumes (Díaz *et al.*, 2017; Malm *et al.*, 2018). This study aimed to explore how drought stress influences phenolic metabolism in three *Vicia faba* L. cultivars (Reinablanca, Aquadulce L., and Alfred) by measuring total phenolics (TP), condensed tannins (CTs), and vanillin-reactive compounds. This comparison seeks to reveal cultivar-specific differences in phenolic responses to drought, providing biochemical support for the role of phenolic metabolism in stress tolerance. Findings from this research are vital for breeding programs focused on developing stress-resistant legume varieties and for understanding the biochemical mechanisms that underpin stress resilience.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Three *Vicia faba* L. cultivars (Reinablanca, Aquadulce L., and Alfred) were used. Plants grew under controlled greenhouse conditions at the Experimental Station of the Faculty of Natural and Life Sciences, Mentouri University, Constantine 1, Algeria. The experiment took place in spring 2025 using a randomized complete block design with two treatments.

- Traditional (T): Plants cultivated under ideal irrigation conditions.
- Selected (S): Plants exposed to controlled drought stress to mimic water deficit.

Each treatment was applied to three biological replicates per cultivar. Plants were grown in 5L pots filled with a mixture of loam, sand, and compost (2:1:1, v/v/v), maintained at $25 \pm 2^\circ\text{C}$ under a 16hour light/8hour dark photoperiod. Drought stress was imposed at the pod-filling stage by withholding water until soil moisture content reached 40% of field capacity. Control plants were kept at 80-90% of field capacity.

After 15 days of treatment, mature seeds were harvested, air-dried, and ground into a fine powder (<1 mm particle size) for chemical analyses.

2.2. Determination of Total Phenolic Content (TP)

TP was determined using the Folin-Ciocalteu method as described by Singleton *et al.* (1999) with slight modifications. Briefly, 0.5 mL of methanolic extract (1 mg/mL) was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. After incubation at 25°C for 30 minutes in the dark, absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM), based on a calibration curve ($R^2 > 0.99$).

2.3. Determination of Butanol-Soluble Phenolic Compounds

Butanol-extractable phenolics, which indicate polymerized phenolic derivatives, were quantified following Porter *et al.* (1986). One milliliter of methanolic extract was combined with 6 mL of butanol-HCl reagent (95:5 v/v) and 0.2 mL of ferric reagent (2% $\text{FeNH}_4(\text{SO}_4)_2$ in 2 M HCl). The mixture was heated at 95°C for 60 minutes, cooled to room temperature, and the absorbance was measured at 550 nm. Results were expressed as mg catechin equivalents per gram of dry matter (mg CE/g DM).

2.4. Determination of Vanillin-Reactive Compounds

Vanillin-reactive compounds, mainly monomeric flavan-3-ols, were measured using the vanillin assay (Broadhurst & Jones, 1978). In short, 1 mL of extract was combined with 5 mL of 1% vanillin in 70% sulfuric acid. The mixture was incubated at 30°C for 20 minutes, and absorbance was read at 500 nm. Results were expressed as mg catechin equivalents per gram of dry matter (mg CE/g DM).

2.5. Extraction Procedure

Phenolic compounds were extracted from finely ground seed powder using 80% methanol (v/v). About 1 g of powder was homogenized in 10 mL of solvent and sonicated for 30 minutes at room temperature.

The mixture was then centrifuged at $4,000 \times g$ for 10 minutes, and the supernatant was collected. The extraction process was repeated twice, and the combined extracts were filtered through Whatman No. 1 paper and stored at 4 °C until analysis.

3. Results and Discussion

3.1. Total Phenols (TP)

The present study examined the impact of drought on the phenolic metabolism of three *Vicia faba* L. cultivars (Reinablanca, Aquadulce L., and Alfred) by measuring total phenolics (TP), condensed tannins (CTs), and vanillin-reactive compounds. Total phenolic content varied from 46.53 mg/g DM in Reinablanca under traditional treatment (T) to 92.68 mg/g DM in Alfred under selected treatment (S), reflecting both varietal differences and treatment effects (Fig. 1). Across all cultivars, the selected treatment significantly increased TP, suggesting that post-harvest selection and controlled processing can boost the accumulation of bioactive compounds, even under environmental stress like drought (Martins *et al.*, 2016; Serafini *et al.*, 2015). Alfred consistently showed the highest TP, indicating a genetic tendency for phenolic buildup. In contrast, Reinablanca, despite its lower baseline TP, showed a notable improvement under selected treatment, highlighting the influence of both genotype and management practices on phenolic metabolism (Aprotosoiaie *et al.*, 2016).

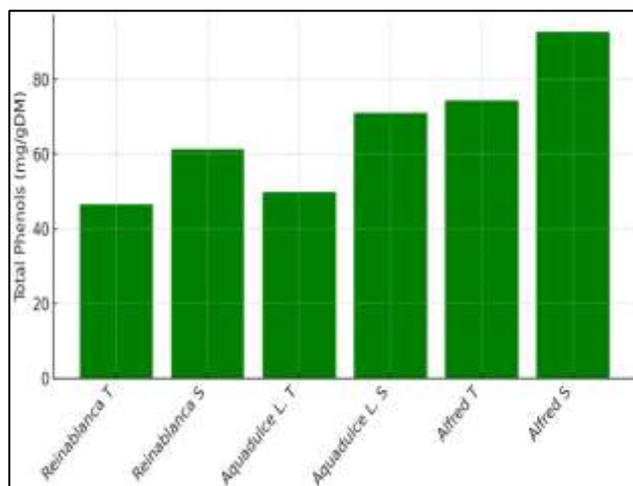


Figure 1. Total phenolic content (mg/g DM) in three *Vicia faba* cultivars under different treatments.

These results show that both genetic background and post-harvest treatments significantly affect phenolic metabolism in *Vicia faba* L. Alfred had the highest TP and butanol levels, indicating a genetic ability to produce phenolic compounds. Reinablanca and Aquadulce, although with lower TP, responded positively to the chosen treatment, suggesting that environmental management can partly offset lower natural phenolic potential. Drought-induced phenolic buildup likely serves as an adaptive stress response. Abiotic stress, such as drought, causes oxidative stress in plants, which can activate the phenylpropanoid pathway, boosting the production of phenolics, condensed tannins, and vanillin precursors (Dixon & Paiva, 1995; Mierziak *et al.*, 2014). The observed increase in TP and butanol under the chosen treatment is therefore consistent with a metabolic adaptation aimed at enhancing antioxidant defenses.

3.2. Butanol Content

Butanol-soluble phenolic compounds, indicative of polyphenol alcohol derivatives, reflected TP trends. Alfred S showed the highest levels (109.35 mg/g DM) (Fig. 2). The positive correlation between TP and butanol content suggests that drought and selection treatments promote the synthesis of soluble polyphenols, boosting antioxidant capacity (Haminiuk *et al.*, 2012). Notably, Aquadulce S, despite having lower TP than Alfred S, exhibited substantial

butanol content (101.66 mg/g DM), indicating cultivar- and treatment-specific differences in phenolic composition and solubility (Strobel *et al.*, 2020).

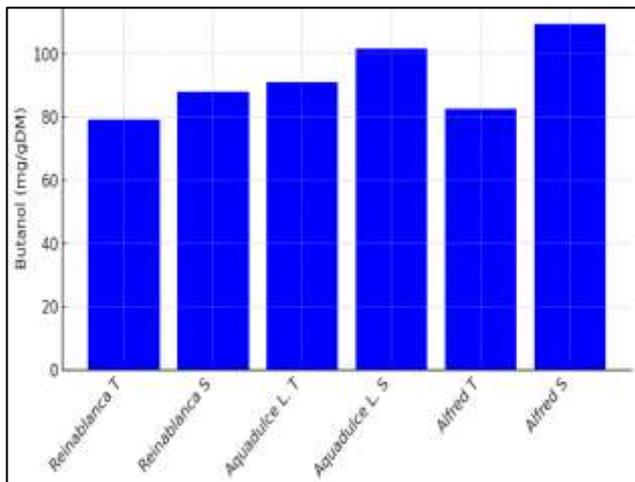


Figure 2. Butanol-extractable phenolic compounds (mg/g DM) in *Vicia faba* cultivars under control and stress conditions.

The butanol-soluble fraction, which includes polymerized phenolics and flavonoid alcohol derivatives, contributes to antioxidant effects and sensory qualities such as astringency and bitterness (Haminiuk *et al.*, 2012; Monagas *et al.*, 2009). The positive correlation with TP suggests that drought and post-harvest selection increase both free and polymerized phenolics, possibly enhancing the functional and nutritional qualities of these cultivars.

3.3. Vanillin Content

Vanillin-reactive compounds showed an opposite trend compared to TP and butanol. Typical treatments generally had higher vanillin levels (e.g., Reinablanca T: 41.40 mg/g DM; Alfred T: 51.21 mg/g DM) than the selected treatments (Fig. 3). This indicates that while TP and condensed tannins increase under selected treatment and drought, vanillin may be partially broken down or diverted during stress-related changes (Afoakwa *et al.*, 2008; Fowler *et al.*, 2020).

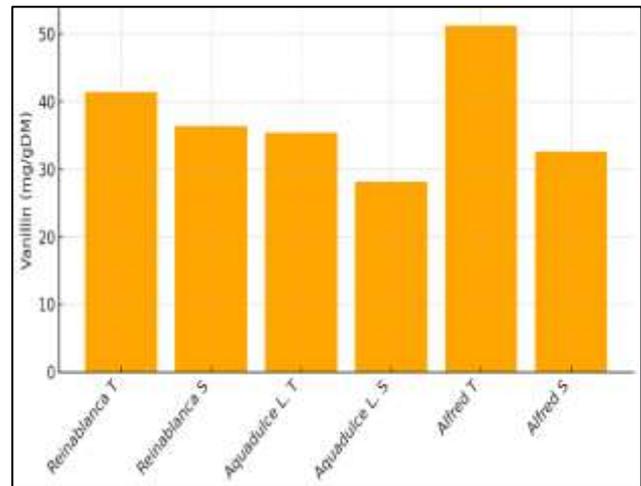


Figure 3. Vanillin-reacting compounds (mg/g DM) in *Vicia faba* cultivars under control and stress conditions.

Vanillin, a key aromatic compound, decreased under selected treatment across all cultivars, indicating a metabolic trade-off: stress-induced phenolic synthesis might divert precursors from volatile flavor compound biosynthesis. Similar observations have been reported in cocoa and other phenolic-rich crops, where treatments that boost polyphenol accumulation are associated with reduced levels of flavor-active compounds (Afoakwa *et al.*, 2008; Fowler *et al.*, 2020).

3.4. Comparative Analysis

Among the three cultivars, Alfred showed the highest bioactive potential, with the highest TP and butanol concentrations. Reinablanca retained higher vanillin content under traditional treatment, indicating better flavor potential. Aquadulce displayed intermediate TP but relatively high butanol content, reflecting distinct phenolic profiles. These differences highlight the combined influence of genotype, drought stress, and post-harvest treatments on phenolic metabolism (Monagas *et al.*, 2009; Martins *et al.*, 2016).

Our findings agree with previous studies showing that environmental factors, including drought, influence phenolic metabolism in legumes, impacting both bioactive composition and sensory qualities (Di Mattia *et al.*, 2014; Serafini *et al.*, 2015). Choosing the right cultivar and managing post-harvest conditions are

therefore essential to balance the levels of health-promoting compounds with desirable organoleptic qualities.

Alfred's high TP and butanol levels under drought stress indicate its potential for use as a functional food and nutraceutical. In contrast, Reinablanca, with higher vanillin content under traditional treatment, may be better suited for flavor-focused products. Aquadulce L. displayed intermediate traits, balancing antioxidant potential and sensory appeal.

These results further support the idea of stress-adaptive metabolic plasticity, in which plants reallocate metabolic resources during stress to boost antioxidant defenses at the expense of specific secondary metabolites, such as flavor-related compounds (Dixon & Paiva, 1995; Mierziak et al., 2014).

4. Conclusion

Drought stress significantly impacts the phenolic metabolism of *Vicia faba* L., with noticeable differences among different cultivars. Alfred showed the highest build-up of total phenols and butanol-soluble compounds under drought conditions, indicating greater adaptive ability and antioxidant capacity. In contrast, vanillin content decreased during stress, reflecting a metabolic shift from flavor-related compounds toward antioxidant production.

These findings emphasize the activation of secondary metabolic pathways as a defense mechanism against oxidative stress. Genotypic differences highlight the importance of genetic factors in influencing metabolic responses. Overall, phenolic composition could serve as a reliable biochemical marker of drought tolerance and metabolic adaptation in faba beans. The Alfred cultivar shows particular potential for breeding programs and functional food applications focused on improving nutritional and antioxidant properties. Further studies combining biochemical and molecular methods are needed to clarify the regulatory mechanisms that control phenolic biosynthesis under drought.

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