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Effect of antifreeze action products used to prevent frost damage during the vegetative and reproductive stages of common bean

Calderón-Tomás, Celene[1]; Díaz-Ruiz, Ramón[1*]; Contreras-Ramos, Juan[1]; Pérez-Ramírez, Efraín[1]; Pérez-Armendáriz, Beatriz[2]; Álvarez-Gaxiola, Jesús F.[1]

[1] Colegio de Postgraduados, Campus Puebla. Boulevard Forjadores de Puebla núm. 205, Santiago Momoxpan, San Pedro Cholula, Puebla. México. C.P. 72760.
[2] Universidad Popular Autónoma del Estado de Puebla. Avenida 9 Poniente 1712, Barrio de Santiago, Puebla, México. C.P. 72090
* Correspondence: dramon@colpos.mx

ABSTRACT

Objective: To establish the frost damage to the stem and root nodules and protection degree of products with antifreeze potential, during the V2, V3, R6, and R7 phenological stages of bean.

Design/Methodology/Approach: Black beans with a type II indeterminate bushy habit were collected. Antifreeze (An), amino acids (Am), gibberellins (Gib) and their combinations were applied at 48-hour intervals. Subsequently, the plants were subjected to frost (0 °C) in a freezer for 1.5 h. Damage was evaluated in a 0 to 100% scale. The nodules were stained with 2,3,5-triphenyl tetrazolium chloride salt. Stained nodules were considered undamaged and non-stained nodules were considered damaged.

Results: Significant differences were found during the phenological stages and between the antifreeze action product treatments. Stage V3 was the most tolerant to frost, while stages R6 and R7 were the most susceptible. The number of undamaged and damaged nodules showed highly significant differences ($p \leq 0.01$) between phenological stages and between treatments, as well as in the total number of nodules. An and Ve+An recorded good effects, followed by Ve+Am. Gib was the least efficient product during the four stages.

Study Limitations/Implications: The increase of substances with antifreeze effect in different doses should be tested.

Findings/Conclusions: Frost caused different levels of damage in each phenological stage. V3 stood out as the most tolerant stage. All the products recorded different protection degrees during the phenological stages. Ve+An, An, and Ve+Am recorded the highest antifreeze action.

Keywords: *Phaseolus vulgaris* L., low temperatures, phenology, antifreeze products.

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INTRODUCTION

As a result of its protein content, bean (*Phaseolus vulgaris* L.) is a staple food worldwide. It helps soil fertilization (Maldonado *et al.*, 2015), through its atmospheric nitrogen fixation. Consequently, bean is grown in different areas, including humid tropics, semi-arid regions (Hernández-López *et al.*, 2013), and highlands, where low temperatures severely damage

production. Frost causes great damages to agriculture, because this weather phenomenon is very difficult to predict. Although implementing a system to protect crops from frost is very expensive, there are several alternatives, including a passive method to prevent the effect of frosts. This long-term method is particularly beneficial under frost conditions and is related to biological and environmental technologies used to reduce potential damage (Snyder and Melo-Abreu, 2010).

One of the passive methods used to prevent frost damages is the use of organic matter and the appropriate type of soil. This method highly improves soil characteristics, increasing heat capacity, maintaining a stable thermal regime, increasing and preserving structural soil stability, permeability, and water retention capacity, facilitating gas exchange, reducing erosion, and improving crop nutrition (Labrador, 2001; Fuentes-Yague, 2002). Another passive mechanism to prevent frost is a good soil nutrition. Consequently, the application of amino acids helps to mitigate wounds caused by abiotic stress. These biostimulants are known for their positive effects on growth and yield (Sadak *et al.*, 2015). Amino acids act when plants suffer physiological changes, playing a very important role in water balance (Espasa-Manresa, 1983). Stabilized vegetable oils can protect plants from temperature changes (extreme cold weather), preventing the formation of intracellular ice. Therefore, the objective of this study was to determine the frost damage in the stem and root nodules and the protection degree of potential antifreeze substances, during the V2, V3, R6, and R7 phenological stages of bean.

MATERIALS AND METHODS

Bean variety and sowing

Black beans with a type II indeterminate bushy habit were collected from the Tepeyahualco de Cuauhtémoc community in the State of Puebla. The seeds were sown in 21 cm wide × 30 cm tall plastic pots, with agricultural soil (As) and vermicomposting (Ve) prepared with cow manure mixed with agricultural soil (50:50 ratio). Seeds were placed at a depth of 2.5 cm and were fertilized that same day, with the ammonium nitrate and triple super phosphate and calcium sources for nitrogen and phosphorous, respectively, using the 40-40 formula.

Phenological stages

The vegetative stages were V2 and V3, while the reproduction stages were R6 and R7. The identification of the beginning of each phenological stage was based on the characteristics established by Escalante-Estrada and Kohashi-Shibata (1993).

Antifreeze action products

The following products were applied to the plant stems: antifreeze (An), amino acids (Am), and gibberellins (Gib). The An was made up of 95% stabilized vegetable oil (Grupo Ibarquim S. A. de C.V.). The thin biodegradable waxy layer produced by the oil acts as a physical barrier during extreme cold weather (≥ 0 °C), preventing the formation of intracellular ice (Table 1). The Am used in the experiment came from the Aminocel 500© commercial product. Amino acids enable the fast generation of proteins, but using less energy. They favor the balance between photosynthesis and respiration. This product was made up of 50% free amino acids, 10% nitrogen, 8% phosphorous, and 10% potassium. Finally, Gib were obtained from BioGib10PS, made up of 10% gibberellic acid and 90% diluents and conditioners. Gib stimulates plant growth, uniforms flowering, and improves fruit bearing and development.

Frost simulation

An, Am, and Gib were applied when the plants reached their vegetative and reproductive stages. Four plants were randomly chosen for the application of each treatment. The plants were subjected to two applications with 48 h intervals. The products were manually applied in the stems, using 900 ml atomizers. The doses applied were those recommended by the manufacturers of each product (Table 1). No products were applied to the AS and Ve control treatments. At 96 h after the application, plants were placed inside a Tor Rey Refrigeration CV-32 freezer and subjected to frost (0 °C) for 1.5 h.

Evaluation of frost damage in the stem

Plants were evaluated 48 h after they were taken out of the freezer. Damages produced to the plant organs were expressed in percentages from 0 to 100%, where 0 is an undamaged plant and 100% is a completely damaged plant.

Evaluation of frost damage in the root

Root damage was evaluated staining the roots with 2,3,5-triphenyl tetrazolium chloride salt. The seed feasibility test proposed by Moreno (1984) was used to stain and detect *Rhizobium* bacterial activity. The roots were placed in beakers and were completely immersed in a 0.1% tetrazolium salt solution for 60 minutes. Subsequently, the red-stained nodules were counted. These nodules were considered as undamaged. Meanwhile, the unstained nodules were considered as damaged.

Data analysis

An ANOVA was performed to detect statistical differences between treatments, while Tukey's Test (α=0.05) was used to determine which treatments better mitigated frost damages. Both analyses were conducted with SAS v.9 statistical package for Windows.

Table 1. Antifreeze action products and doses used in the experiment.

Treatments	Dosage	Abbreviation
Agricultural soil	--	AS
Antifreeze	10 ml l^{-1}	An
Amino acids	1.5 g l^{-1}	Am
Gibberellins	0.05 g l^{-1}	Gib
Vermicompost*	50%	Ve
Vermicompost*+Antifreeze	50%+10 ml l^{-1}	Ve+An
Vermicompost*+Amino acids	50%+1.5 g l^{-1}	Ve+Am
Vermicompost*+Gibberellins	50%+0.05 g l^{-1}	Ve+Gib

RESULTS AND DISCUSSION

Stem damage caused by frost

The analysis of variance detected significant differences ($p \leq 0.01$) in the bean stem damage caused by frost, during the different phenological stages and between antifreeze action product treatments. Stage V3 (Figure 1) recorded the lowest frost damage (7.5%), while stages V2, R6, and R7 reached 22-31% damage to the stem. Consequently, V3 is the most tolerant stage to low temperatures. Meanwhile, the different damage level caused to other stages can be the result of the susceptibility of each stage to frost. Ambroise *et al.* (2020) mentioned that frost tolerance depends on the species, type of plant organ, and plant age; consequently, the earliest stages are more tolerant to frost. For their part, Calderón-Tomás *et al.* (2023) studied frost damage during the vegetative stage of bean and recorded that V3 was the most tolerant stage. Therefore, reproduction is the most vulnerable stage, given the susceptibility of reproductive organs to low temperatures.

The treatments showed a different protection degree in each stage (V2, V3, R6, and R7). During stage V2, damage was not detected with the Ve+An, Ve+Am, Am and An treatments (Figure 2), while the highest damage percentage was recorded with the Gib (71%) and Ve+Gib (83%) treatments. This situation can be the result of the exogenous application of gibberellins, which produces a wide variety of responses during the development of the plant, particularly at cellular level (Amador-Alférez *et al.*, 2013). Damage was lower (10%) during stage V3. However, the Ve+Gib treatment resulted in 50% stem damage. The significant damage suffered by Gib-treated plants during these stages can be the consequence of the impact of low temperatures on the synthesis and transport of hormones (Jones, 1985), which causes a physiological imbalance. The lowest frost damage was recorded during the reproductive stage of bean, with the Ve and Am treatments (Figure 2), while the highest damage was recorded with AS, followed by Gib. The Ve+An treatment applied in plants during stage R7 recorded a higher protection degree, followed by An. Meanwhile, the highest damage was recorded by the Ve+Gib treatment.

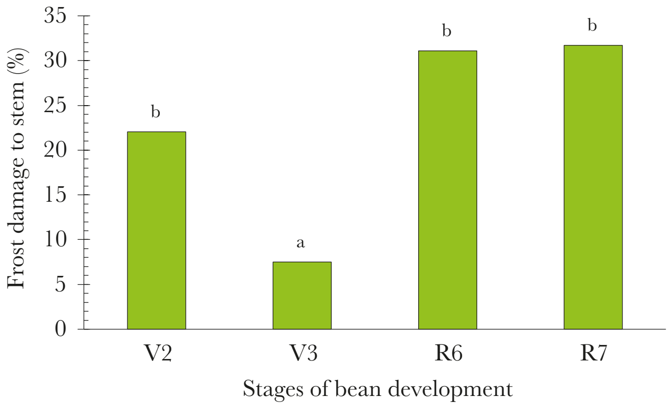

Figure 1. Frost damages in bean stems, during their phenological, vegetative, and reproductive stages.

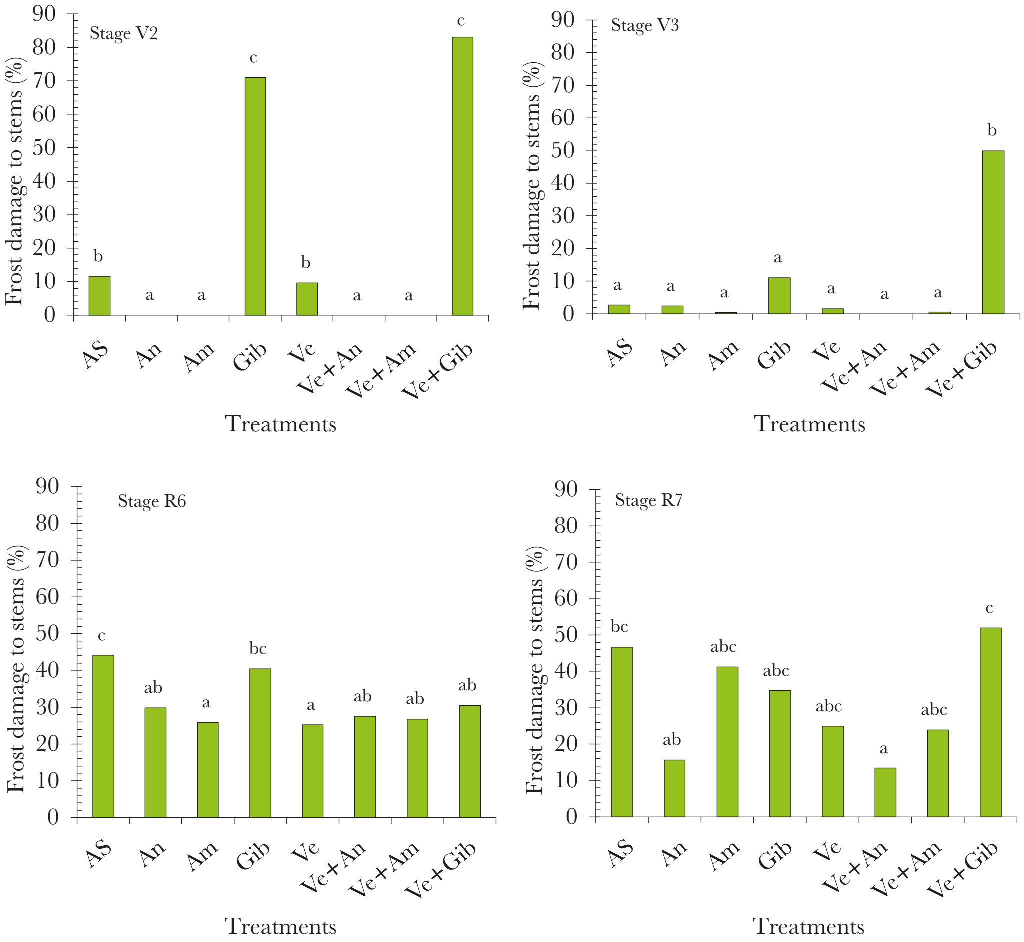

Figure 2. Frost damage to stems with the application of antifreeze products in the vegetative and reproductive stages of beans. AS: agricultural soil. An: antifreeze. Am: amino acids. Gib: gibberellins. Ve: vermicompost. Ve+An: vermicompost+antifreeze. Ve+Am: vermicompost+amino acids. Ve+Gib: vermicompost+gibberellins.

Each organ of the plant showed different sensitivity to the same frost conditions, but the leaves recorded most of the damage. This phenomenon could be related to the water percentage (90-94%) of pulses (Alcántar-González and Trejo-Téllez, 2009). Except for the Ve+Gib treatment, the lowest damage to the leaf structures in stages R6 and R7 was recorded in bean plants whose treatment included vermicompost. The water retention of vermicompost helped to alleviate the damage caused by low temperatures, because the roots were still able to provide water to the stem. Meanwhile, Badaruddin and Meyer (2001) worked with fields at full capacity and reported a lower damage to alfalfa and soybean seedlings caused by frost in soils with light texture than to seedlings that grew in soils with heavy texture. Therefore, the water content resulting from the moisture in the roots softens the impact of frosts. In conclusion, substrates with good water retention capacity (*e.g.*, compost or vermicompost) help to diminish the damages caused by low temperatures.

Undamaged leaves were protected by the antifreeze in the Ve+An treatment; meanwhile, the protection provided by the Ve+Am and Am treatments could be the result of an increase in the nutritional reserves of plant tissues, which lessen the effect of frosts. According to Chaar (2013), nutrient reserves influence the resistance to frosts, through the starch degradation in osmotically active compounds. This phenomenon increases the over-freezing capacity of the plant tissue and prevents or diminishes the risk of freezing.

Gibberellins are synthesized in different parts of the plants. This hormone is found in high levels on the leaves and buds of seedlings during their growing stages (Jordán and Casaretto, 2006). Therefore, the clear division and cell elongation of constantly growing tissues could favor the conditions under which frosts directly impact the tissues. Additionally, low temperatures determine the conversion of inactive gibberellins into active molecules in the other organs (Jordán and Casaretto, 2006).

Shafiq *et al.* (2012) reported that, during the flowering and pod development stages, the seed yield of peas sown on the field was negatively impacted by their exposition to −4.8 °C for 4 h. This phenomenon could be observed in pod, flower, and bud abortions and deaths, as well as the smaller seed size. The said research team concluded that genetic variation was reported in 83 accessions collected in 34 countries, 60 of which did not have buds, flowers, or pods after the frosts.

Root

Significantly high differences ($p \leq 0.01$) in the number of undamaged (NodU) and damaged (NodD) nodules were recorded between phenological stages and between treatments. The total number of nodules was highly significant between stages and treatments (Table 2).

More nodules were formed during stages R6 and R7 (Figure 3). Meanwhile, a lower number were recorded in stages V2 and V3, when a lower number of damaged nodules was also observed.

The highest number of nodules was developed in stage V2 with Ve+An (Figure 4). The AS treatment recorded the highest number of NodD. The Ve+An and Ve+Am treatments recorded the highest number of undamaged nodules in stage V3; the number of damaged nodules was not significantly different between treatments in this stage. Stage R6 recorded the highest number of undamaged nodules with the Am treatment, while the lowest quantity was obtained with the Ve+An treatment. For its part, the Gib treatment provided the highest number of NodD, while the Am, Ve, and Ve+An treatments recorded the lowest quantity. The highest number of damaged nodules was recorded in stage R7

Table 2. Mean squared error of the number of nodules developed by the roots of bean plants subjected to frost.

Source	NodU	NodD	NodTotal
Sta	2071202.642**	225.7862319**	2235285.961**
Tre	57929.212**	64.4558377*	49046.524**
Sta*Tre	58937.962**	41.4145223**	58704.060**

ns: non-significant. ** significantly statistical differences ($p \leq 0.01$). Sta: stage. Tre: treatments. NodU: number of undamaged nodules. NodD: number of damaged nodules. NodTotal: total number of nodules.

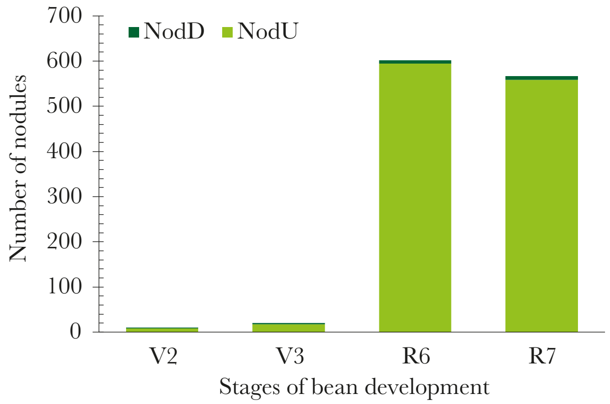

Figure 3. Number of undamaged and damaged nodules during four phenological stages of common bean. NodU: number of undamaged nodules. NodD: number of damaged nodules. Tukey ($p \leq 0.05$). Different letters represent a significant difference.

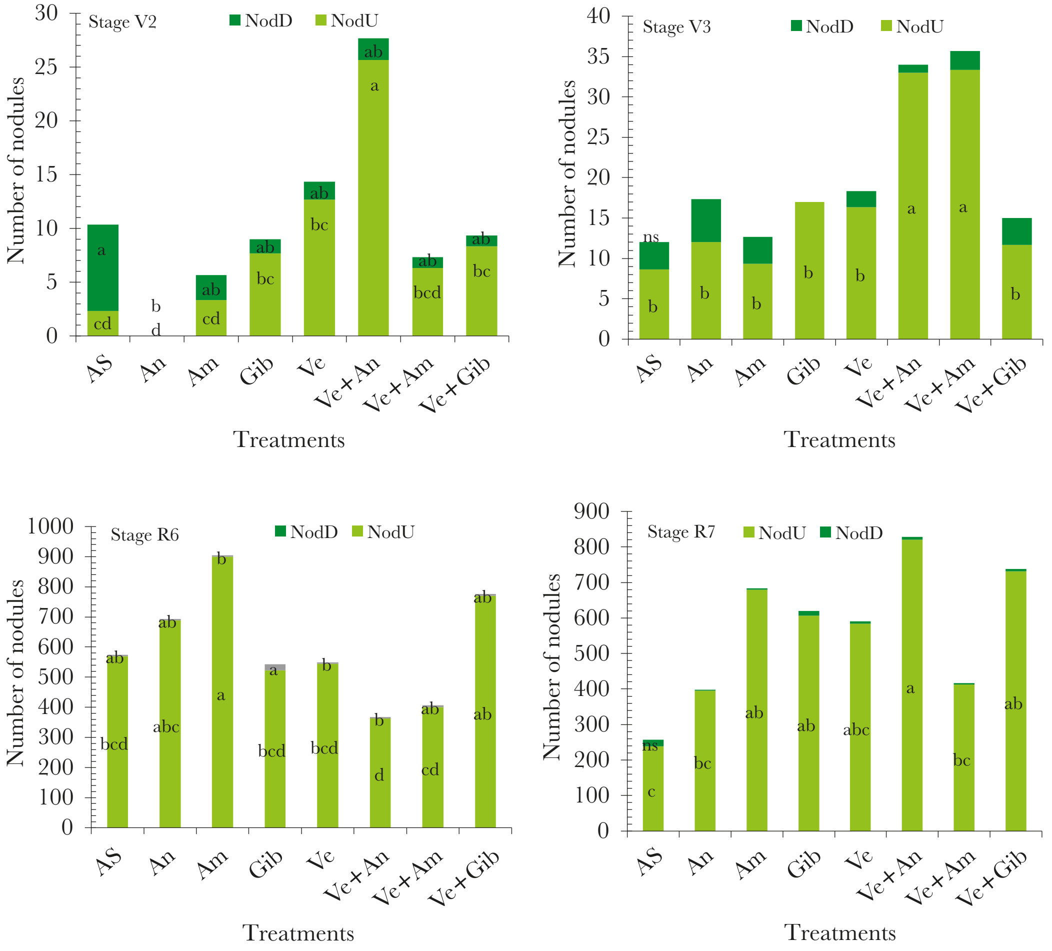

Figure 4. Number of root nodules damaged and undamaged by frosts, as a consequence of the application of antifreeze products, during four phenological stages of common bean. Tukey ($p \leq 0.05$). Different letters represent significant statistical differences. NodU: number of undamaged nodules. NodD: number of damaged nodules. AS: agricultural soil. An: antifreeze. Am: amino acids. Gib: gibberellins. Ve: vermicompost. Ve+An: vermicompost+antifreeze. Ve+Am: vermicompost+amino acids. Ve+Gib: vermicompost+gibberellins.

with the Ve+An treatment and the lowest number was reported with AS; meanwhile, the amount of NodD did not show a significant difference. A greater number of undamaged nodules and a lower number of damaged nodules mean that the root was not damaged by the frost; therefore, this organ of the bean plant can play an important role in the recovery of plants in which the treatments resulted in a lower damage to the stem. The final survival and the shoot production in *Vicia faba* grown in pots and exposed to freezing temperatures was related to the survival of the roots, rather than to the survival of the shoots (Sallam *et al.*, 2015). A similar phenomenon could happen with beans. Despite an increase in the global mean temperature, frost damage is likely to increase in the future, mainly because a greater climate variability would expose roots to lower temperatures and to more frequent freezing and unfreezing cycles, which could diminish agricultural productivity in temperate and colder climates (Ambroise *et al.*, 2020).

CONCLUSIONS

The impact of frosts on plant stems during the different evaluation stages caused damage of various magnitudes. Stage V3 was the most tolerant to frost. Antifreeze product treatments had a different effect in each stage: the Ve+An, Am, and Ve treatments provided greater protection during stage V2; the Ve+An treatment did not record damages in the stems as a consequence of the frost; the Ve+Gib treatment reported the highest damage; and the best treatments during stage R6 and R7 were the Ve and An treatments, respectively.

Frosts did not have a visible effect on the roots. However, the number of nodules was statistically different between treatments: the Ve+An treatment recorded the highest number of active nodules in stage V2, while the Ve+An and Ve+Am treatments had an equivalent effect in stage V3. For their part, the Am and Ve+An treatments reported the highest number of active nodules in R6 and R7, respectively.

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