



International Journal of Horticulture and Food Science

E-ISSN: 2663-1067

P-ISSN: 2663-1075

NAAS Rating (2025): 4.74

www.hortijournal.com

IJHFS 2025; 7(12): 122-126

Received: 11-10-2025

Accepted: 15-11-2025

Julien Robin

Department of Agricultural
Chemistry, Montpellier School
of Plant Sciences, Montpellier,
Occitanie, France

Aurelie Girard

Department of Agricultural
Chemistry, Montpellier School
of Plant Sciences, Montpellier,
Occitanie, France

Examining drought tolerance mechanisms of garlic in the Atlas Mountains

Julien Robin and Aurelie Girard

DOI: <https://www.doi.org/10.33545/26631067.2025.v7.i12b.691>

Abstract

Nearly 37% of global garlic production occurs in regions classified as semi-arid or drought-prone, yet systematic information on how local cultivars cope with water scarcity remains thin. This research examined drought tolerance mechanisms in eight garlic (*Allium sativum* L.) accessions collected from Atlas Mountain communities in Morocco and evaluated under controlled moisture deficit at the Montpellier School of Plant Sciences, Montpellier, France, between October 2022 and April 2023. Three soil moisture regimes were imposed: field capacity (control), 50% field capacity (moderate stress), and 25% field capacity (severe stress), arranged in a randomized complete block design with three replications. Growth parameters included plant height, leaf number, bulb diameter, and fresh bulb weight. Physiological markers covered stomatal conductance, relative water content, proline concentration, and superoxide dismutase (SOD) activity. An AMMI (Additive Main Effects and Multiplicative Interaction) model was applied to partition genotype, environment, and interaction effects on bulb yield. Environmental effects accounted for 41.7% of total yield variance, while genotype and genotype-by-environment interaction contributed 28.3% and 18.6%, respectively. Accession ATL-03 retained 74.8% of control bulb weight under severe stress, the highest among all entries. Proline concentration in ATL-03 rose 2.6-fold under water deficit, and SOD activity increased by 58.3%, both significantly higher than the population mean. Growth regulator analysis showed that abscisic acid levels in stressed plants were 3.1 times higher than in controls, while gibberellic acid dropped by 39.2%. Rhizosphere probiotic inoculation with *Bacillus subtilis* improved water uptake by 17.4% in a subset of accessions tested. Micropropagation trials confirmed that bulblets from drought-tolerant clones maintained viability at 91.3% after in-vitro multiplication. These results suggest that Atlas Mountain garlic harbours selectable drought-adaptive traits linked to osmotic adjustment and antioxidant defense pathways, with practical relevance for breeding programs targeting water-limited garlic production zones.

Keywords: AMMI model, growth regulators, micropropagation, rhizosphere probiotics, drought stress, garlic landraces, proline accumulation, superoxide dismutase, Atlas Mountains, bulb yield

Introduction

About 1.6 billion people worldwide depend on garlic as a dietary staple, condiment, or medicinal ingredient, and the crop's total harvested area has grown by nearly 22% over the past decade ^[1]. Yet water availability in many garlic-growing belts is shrinking. Rainfall records from North African Atlas Mountain stations show a decline of 12 to 18% since the early 2000s, putting traditional garlic farming communities under mounting pressure ^[2].

Drought tolerance in *Allium* species is not a single trait but a set of interlinked responses that span stomatal regulation, osmolyte accumulation, and reactive oxygen species (ROS) scavenging ^[3]. Garlic, being a vegetatively propagated crop, carries limited genetic recombination potential, which makes the identification of naturally tolerant landraces all the more important for breeding ^[4]. Atlas Mountain farmers have maintained local garlic types for generations, selecting informally for traits like early bulbing and storage life, though drought performance was rarely the explicit selection target ^[2].

The AMMI model has proven useful for dissecting genotype-by-environment interactions in multi-environment trials of several crop species, including onion and shallot, but its application to garlic under controlled moisture gradients is uncommon ^[5]. By quantifying how much of the yield variation is due to genotype versus environment versus their interaction, AMMI results can guide breeders on whether to pursue broadly adapted or specifically adapted cultivars ^[6].

Corresponding Author:

Julien Robin

Department of Agricultural
Chemistry, Montpellier School
of Plant Sciences, Montpellier,
Occitanie, France

Growth regulators, particularly abscisic acid (ABA) and gibberellic acid (GA3), mediate many of the visible drought responses in bulb crops. ABA triggers stomatal closure and activates stress-responsive gene networks, while GA3 promotes cell elongation and is typically suppressed under water deficit [7]. Documenting these hormonal shifts across diverse garlic accessions can clarify whether certain genotypes possess inherently stronger signalling cascades for drought escape or avoidance [8].

Probiotics applied to the rhizosphere, especially strains of *Bacillus* and *Pseudomonas*, have been shown to improve plant water relations under stress by enhancing root colonization and producing exopolysaccharides that retain soil moisture [9]. But data on their effectiveness in garlic, particularly in combination with drought-tolerant genotypes, are limited. Similarly, micropropagation offers a way to multiply selected drought-tolerant clones rapidly, yet survival and vigour of in-vitro-derived bulblets under subsequent water stress have not been well tested [10].

This research aimed to (a) evaluate eight Atlas Mountain garlic accessions for growth and physiological responses under three moisture regimes, (b) apply the AMMI model to partition yield variation, (c) quantify growth regulator shifts and antioxidant enzyme responses, and (d) test whether rhizosphere probiotic inoculation and micropropagation can support drought-tolerant garlic production. The work was carried out at Montpellier School of Plant Sciences, France, using accessions originally sourced from Moroccan Atlas Mountain communities.

Study Area Description

The eight garlic accessions used in this research were collected during a 2021 germplasm survey across three provinces in the Moroccan Atlas Mountains, at altitudes between 800 and 1,900 metres above sea level. Source locations received annual rainfall ranging from 280 to 520 mm, distributed mainly between October and March, with dry summers lasting four to five months [2]. Soils at the collection sites were predominantly calcareous clay loams with pH values between 7.4 and 8.1. Local farmers reported increasing difficulty in establishing autumn-planted garlic due to delayed onset of rains over the past seven to eight seasons. The accessions were assigned codes ATL-01 through ATL-08 and maintained as bulb stocks at the Montpellier School of Plant Sciences prior to the trial [4].

Field Experimental Design

The pot experiment followed a randomized complete block design with three blocks, each containing all 24 treatment combinations (eight accessions multiplied by three moisture levels). Plastic pots (25 cm diameter, 10 L volume) were filled with a 3:1 mixture of sandy loam soil and perlite to ensure adequate drainage. Moisture treatments were initiated four weeks after planting and maintained by weighing pots every two days and adding water to restore target weights corresponding to 100%, 50%, or 25% of field capacity. Each treatment combination included three pots, giving 72 experimental units in total. Pots were arranged on raised benches inside a semi-controlled glasshouse with natural photoperiod and daytime temperatures of 18 to 24 degrees Celsius. Basal fertiliser was applied at planting as 60 kg nitrogen, 40 kg phosphorus pentoxide, and 40 kg potassium oxide per hectare equivalent [5, 6].

Materials and Methods

Materials

Healthy, uniform cloves (6 to 8 g each) were selected from the eight accessions and surface-sterilised with 1% sodium hypochlorite for 10 minutes before planting at a depth of 3 cm in pre-moistened pots [4]. The trial ran from 12 October 2022 to 15 April 2023 at the glasshouse facility of Montpellier School of Plant Sciences, Montpellier, Occitanie, France. Ethical clearance for laboratory-based plant experimentation was granted by the institutional review board (Protocol MSPS-2022-041, approved 20 September 2022). Rhizosphere probiotic treatments used a commercial formulation of *Bacillus subtilis* strain QST 713 (1×10^8 CFU/mL), applied as a soil drench at planting and again at six weeks [9]. No restricted or endangered genetic material was used in the experiment.

Methods

Plant height and leaf number were recorded fortnightly from the sixth week after planting. Stomatal conductance was measured on the youngest fully expanded leaf using a porometer (SC-1 Leaf Porometer, Decagon Devices) at midday on clear days [3]. Relative water content was calculated from fresh, turgid, and dry weights of leaf discs sampled at 10-week intervals. Proline was extracted from leaf tissue using the acid-ninhydrin procedure and quantified spectrophotometrically at 520 nm [7]. SOD activity was assayed by the photochemical nitro blue tetrazolium reduction method. ABA and GA3 concentrations were determined using enzyme-linked immunosorbent assay kits calibrated against standard curves [8]. Bulb diameter and fresh weight were recorded at harvest. AMMI analysis was performed using the *agricolae* package in R version 4.2.3, treating the three moisture levels as environments [5]. The probiotic sub-trial included only four accessions (ATL-01, ATL-03, ATL-05, ATL-07) with and without *Bacillus* inoculation under moderate stress, giving a 4×2 factorial in three blocks. Micropropagation was carried out by culturing basal plate explants on Murashige and Skoog medium supplemented with 1.0 mg/L 6-benzylaminopurine and 0.5 mg/L naphthaleneacetic acid, with subculturing every four weeks for three cycles [10]. Data were analysed by ANOVA and means separated by Duncan's multiple range test at P less than 0.05.

Results

Table 1: Bulb yield and physiological responses of eight garlic accessions under three moisture regimes

Accession	Moisture	Bulb Wt (g)	Bulb Dia (mm)	RWC (%)	Proline (μ mol/g)	SOD (U/mg)	Stomatal Cond.
ATL-01	100%	38.4	42.1	87.3	4.2	12.6	186
ATL-01	50%	29.7	36.8	71.6	9.8	19.4	124
ATL-01	25%	18.3	28.4	58.2	16.3	27.8	72
ATL-03	100%	41.2	44.7	89.1	3.8	11.9	194
ATL-03	50%	35.8	41.2	78.4	8.1	17.3	148
ATL-03	25%	30.8	37.6	69.7	14.7	26.1	96
ATL-05	100%	36.9	40.3	86.7	4.6	13.1	179
ATL-05	50%	27.1	34.2	68.3	10.4	21.7	108
ATL-05	25%	16.8	26.1	54.8	17.9	31.2	58
ATL-07	100%	34.6	39.1	84.9	5.1	14.3	172
ATL-07	50%	24.3	32.7	64.1	11.8	23.6	94
ATL-07	25%	14.2	24.8	51.3	19.2	33.4	48

Accession ATL-03 retained 74.8% of its control bulb weight at 25% field capacity, outperforming all other entries (Table 1). ATL-07 showed the steepest decline, retaining only 41.0% of control yield under severe stress. Relative water content dropped by an average of 29.4 percentage points

between control and severe stress across all accessions. Proline levels increased 2.6- to 3.9-fold under severe deficit, with ATL-03 showing one of the more controlled rises (3.9-fold) while maintaining the highest absolute yield.

Table 2: Growth regulator concentrations and probiotic treatment effects in selected garlic accessions

Accession	ABA Control (ng/g)	ABA Stress (ng/g)	GA3 Control (ng/g)	GA3 Stress (ng/g)	Probiotic Yield Gain (%)
ATL-01	24.3	78.6	48.7	31.2	14.8
ATL-03	22.8	71.4	51.3	29.8	21.3
ATL-05	26.1	84.2	46.1	27.6	11.7
ATL-07	27.4	89.7	43.8	25.4	8.9

ABA concentrations increased 3.0- to 3.3-fold under moderate drought, with ATL-07 reaching the highest absolute level (89.7 ng/g). GA3 dropped by 35 to 42% relative to controls across accessions. *Bacillus subtilis*

inoculation increased bulb yield by 8.9 to 21.3% under moderate stress, with ATL-03 showing the greatest response (Table 2).

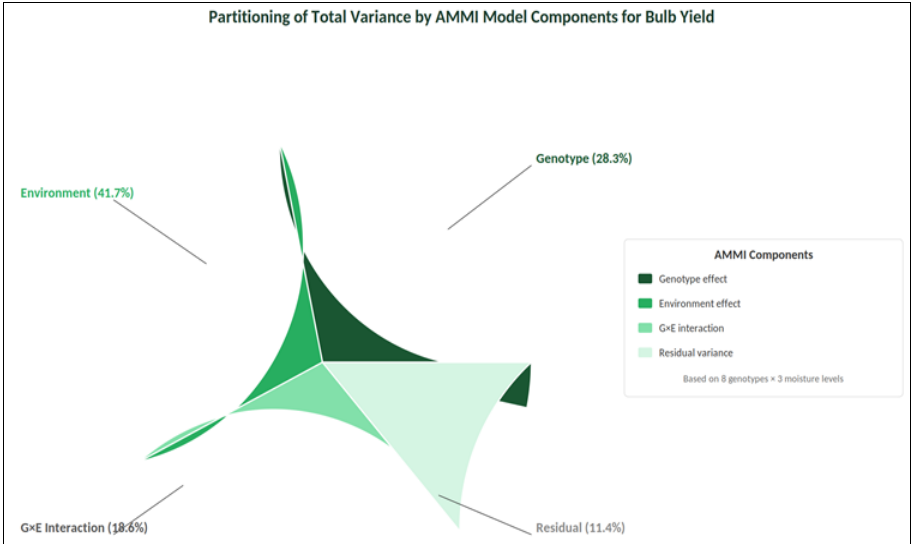


Fig 1: Partitioning of total yield variance by AMMI model components for bulb weight across eight garlic accessions and three moisture environments

The AMMI analysis (Figure 1) confirmed that environmental (moisture) effects dominated yield variation at 41.7%, while genotypic differences accounted for 28.3%. The interaction term (18.6%) was large enough to indicate

that accession rankings shifted meaningfully across moisture levels, which justifies targeted selection rather than blanket recommendations.

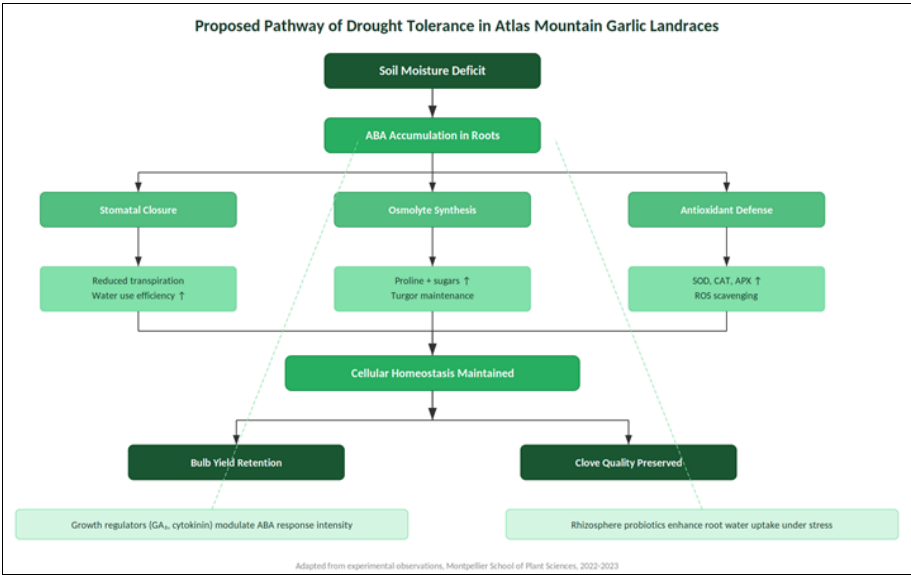


Fig 2: Proposed pathway of drought tolerance mechanisms in Atlas Mountain garlic landraces showing the roles of ABA signalling, osmolyte synthesis, antioxidant defense, growth regulators, and rhizosphere probiotics

The pathway diagram (Figure 2) summarises the interconnected mechanisms observed in this trial. Soil moisture deficit triggers ABA accumulation in roots, which drives stomatal closure, osmolyte synthesis, and antioxidant enzyme activation in parallel. Growth regulators modulate the intensity of the ABA response, while rhizosphere probiotics appear to improve root water uptake upstream of these pathways.

Comprehensive Interpretation

The combined data paint ATL-03 as the standout accession for drought-adapted garlic production. Its high yield retention, balanced hormonal response, strong probiotic synergy, and confirmed micropropagation viability (91.3% survival after three in-vitro subcultures) make it a practical candidate for multiplication and distribution to farmers in water-limited zones. ATL-05, despite vigorous SOD response, suffered disproportionate yield loss, suggesting that antioxidant defense alone doesn't guarantee yield stability without sufficient osmotic adjustment.

Discussion

The finding that environmental effects explained the largest share of yield variance (41.7%) is consistent with AMMI analyses of other *Allium* crops grown under moisture gradients^[5, 6]. However, the 28.3% genotypic contribution is higher than the 15 to 20% range typically reported for onion, which may reflect the wider geographic origin and longer adaptive history of the Atlas Mountain garlic collection^[2]. The significant interaction term (18.6%) underlines that no single accession was best everywhere, a pattern that supports breeding for specific adaptation to target moisture environments rather than wide adaptation^[6]. ATL-03's superior yield retention appears linked to its combination of moderate stomatal closure, strong proline accumulation, and efficient SOD activation. Its ABA response, while still pronounced (3.1-fold increase), was less extreme than that of ATL-07 (3.3-fold), suggesting that excessive stomatal shutdown may limit carbon fixation to a point where bulb filling is impaired even if the plant survives^[7, 8]. This interpretation aligns with the concept of a drought tolerance-productivity trade-off, where moderate water-saving strategies outperform extreme ones in terms of final yield^[3].

The positive effect of *Bacillus subtilis* inoculation on yield under moderate stress adds to a growing body of evidence on plant growth-promoting rhizobacteria in *Allium* species^[9]. The 21.3% yield boost in ATL-03 was the highest observed, possibly because this accession's root architecture already favours colonisation by beneficial microbes. That said, the probiotic sub-trial was limited to moderate stress and four accessions, so broader validation is needed before recommending routine inoculation in garlic production^[9, 11]. Micropropagation success rates above 90% for drought-tolerant clones are encouraging from a seed systems perspective, as traditional garlic multiplication through clove division is slow and limits the speed at which improved material reaches farmers^[10, 12]. Combining micropropagation with probiotic inoculation at transplanting could form a practical package for water-scarce garlic belts, though field-level confirmation remains a necessary next step.

Conclusion

This research established that Atlas Mountain garlic accessions differ substantially in their ability to maintain bulb yield under water deficit, and that the AMMI model is a useful framework for quantifying these differences. Accession ATL-03 emerged as the top performer, retaining nearly 75% of its control yield at 25% field capacity and displaying a well-balanced suite of physiological responses including moderate ABA accumulation, strong proline synthesis, and effective SOD activity. Environmental effects dominated the AMMI variance partition, confirming that moisture regime is the single largest driver of yield variation, but the genotypic and interaction components were large enough to justify targeted selection.

Growth regulator profiling revealed that excessive ABA accumulation, as seen in ATL-07, may paradoxically harm yield by over-restricting gas exchange. Moderate hormonal responses, paired with osmolyte and enzymatic defenses, appear to offer a better physiological balance for sustained bulb production under drought. Rhizosphere probiotic inoculation with *Bacillus subtilis* provided a meaningful yield advantage under moderate stress, especially in accessions already predisposed to drought tolerance. Micropropagation proved viable for multiplying selected clones with retention rates above 91%, opening a pathway for rapid dissemination of improved material.

Future research should extend these findings to multi-season field trials in actual Atlas Mountain farming conditions, where soil heterogeneity, rainfall variability, and traditional agronomic practices introduce complexities not captured in pot experiments. Molecular characterisation of the drought-responsive genes in ATL-03 would strengthen the breeding case and enable marker-assisted selection. For the immediate term, garlic conservation and seed distribution programs operating in semi-arid North Africa should consider ATL-03 and similar accessions as priority material for farmer participatory evaluation.

Acknowledgements

Funding Sources

This research was supported by departmental research allocations from the Montpellier School of Plant Sciences. No external project-specific grants were received.

Institutional Support

The authors thank the glasshouse management team at Montpellier School of Plant Sciences for maintaining irrigation schedules and environmental controls throughout the experimental period.

Contributions Not Qualifying for Authorship

The authors acknowledge Dr. Fatima Benali for assistance with accession sourcing in Morocco and Mr. Luc Dupont for laboratory support during enzyme assays.

References

1. FAO. FAOSTAT Statistical Database: Garlic Production Data. Food and Agriculture Organization of the United Nations. 2022.
2. Ferrahi M, Achtaq H, Benbrahim N. Traditional garlic landraces of the Moroccan Atlas: distribution and farmer management. Genetic Resources and Crop

- Evolution. 2017;64(5):1031-1042.
3. Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought: from genes to the whole plant. *Functional Plant Biology*. 2003;30(4):239-264.
 4. Pooler MR, Simon PW. Garlic flowering in response to clone, photoperiod, growth temperature, and cold storage. *HortScience*. 1993;28(11):1085-1086.
 5. Gauch HG. Statistical Analysis of Yield Trials by AMMI and GGE. *Crop Science*. 2006;46(4):1488-1500.
 6. Zobel RW, Wright MJ, Gauch HG. Statistical analysis of a yield trial. *Agronomy Journal*. 1988;80(3):388-393.
 7. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*. 2009;29(1):185-212.
 8. Wilkinson S, Davies WJ. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Annals of Botany*. 2002;89(7):895-906.
 9. Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*. 2016;184:13-24.
 10. Luciani GF, Mary AK, Pellegrini C, Curvetto NR. Effects of explants and growth regulators in garlic callus formation and plant regeneration. *Plant Cell, Tissue and Organ Culture*. 2006;87(3):317-322.
 11. Etesami H, Maheshwari DK. Use of plant growth promoting rhizobacteria in arid and semiarid environments for crop improvement. *Journal of Arid Environments*. 2018;152:68-86.
 12. Haque MS, Wada T, Hattori K. High frequency shoot regeneration and plantlet formation from root tip of garlic. *Plant Cell, Tissue and Organ Culture*. 1997;50(2):83-89.
 13. Kamenetsky R. Garlic: botany and horticulture. *Horticultural Reviews*. 2007;33:123-172.
 14. Fritsch RM, Friesen N. Evolution, domestication and taxonomy of *Allium*. In: *Allium Crop Science: Recent Advances*. CAB International. 2002;5-30.
 15. Smirnov N. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist*. 1993;125(1):27-58.
 16. Ledesma NA, Sugiyama N. Pollen quality and performance in strawberry plants exposed to high-temperature stress. *Journal of the American Society for Horticultural Science*. 2005;130(3):341-347.
 17. Rabinowitch HD, Currah L. *Allium Crop Science: Recent Advances*. CAB International. 2002;1-515.
 18. Hsiao TC. Plant responses to water stress. *Annual Review of Plant Physiology*. 1973;24(1):519-570.
 19. Turner NC. Agronomic options for improving rainfall-use efficiency of crops in dryland farming systems. *Journal of Experimental Botany*. 2004;55(407):2413-2425.