Conventional breeding of alfalfa for drought-tolerant in limited-water environments

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As climate change intensifies water scarcity, plant breeding for water-limited environments has increasingly focused on developing crop varieties with improved drought tolerance and enhanced water-use efficiency (WUE). In this study, drought stress is defined as any suboptimal yield caused by the crop's inability to meet its evapotranspiration demand.

The objective was to compare the performance of different alfalfa populations under natural, limited-water conditions.

Materials and Methods

Santiago del Estero (SE), NW Argentina
Climate: mesothermal, semi-arid type
Soil: silty loam texture;
Water holding capacity **(WC)** at 2m: 381mm
Climate average 2023/2025:

- Temperature mean annual: min.14.2°C; max.29.1°C
- Rainfall mean annual: 515 mm
- Evapotranspiration reference **(ETo)**≈1300 mm

Alfalfa populations (Pop)

The **Genetic Base (GB)**, serving as the Mediterranean reference population, was developed by intercrossing three cultivars/landraces selected through multi-environment evaluations for their contrasting adaptive traits. It was subsequently evaluated as half-sib progenies in SE and five additional environments (a).

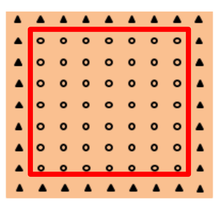
Phenotypic selection 1 (PS1) was obtained from a pool of 122 plants selected after a three-year evaluation (2015–2018 at SE), followed by intercrossing in cages with honeybees at INTA Manfredi (MA) in 2019.

Phenotypic selection 2 (PS2): was derived from 40 plants selected from PS1, evaluated under shelter without irrigation, and intercrossed in cages at MA in 2021.

Salinera INTA (Sal): is a local cultivar used as a tester due to its known adaptability to SE conditions

Experimental design

Complete block, five rep.(4x5)
49 plants/plot; 10 cm between plants



Minimal irrigation was provided during establishment.

Transplanting (Sept.23)

Measurements

By the first harvest in Dec. 2023 (discarded for analysis), an average of 40 (±2) plants per plot was recorded (≈82 pl.m^{-2}). No further irrigation was applied thereafter.

Fifteen harvests were made to measure dry matter (DM) **yield, plant density and plant survival.**

A root zone water balance at two depths (0.60 and 2 m) was computed using these data to estimate **Water Use (WU)** or **ET actual (ETa)** for the whole site, and **Water Use Efficiency (WUE)** was calculated as the ratio between the total DM yield of each population and the total seasonal **ETa**, measured during 2024–2025 at a depth of 2 m.

Statistical analysis

For yield and plants: repeated-measures analysis over time using GLM models: Pop and harvest as a fixed factor and, block and rep (plots) as random factors. At 15th harvest plant survival and density were analyzed separately. Means were compared using the LSD Fisher test (P<0.05).

Results

General trends of yield and water condition

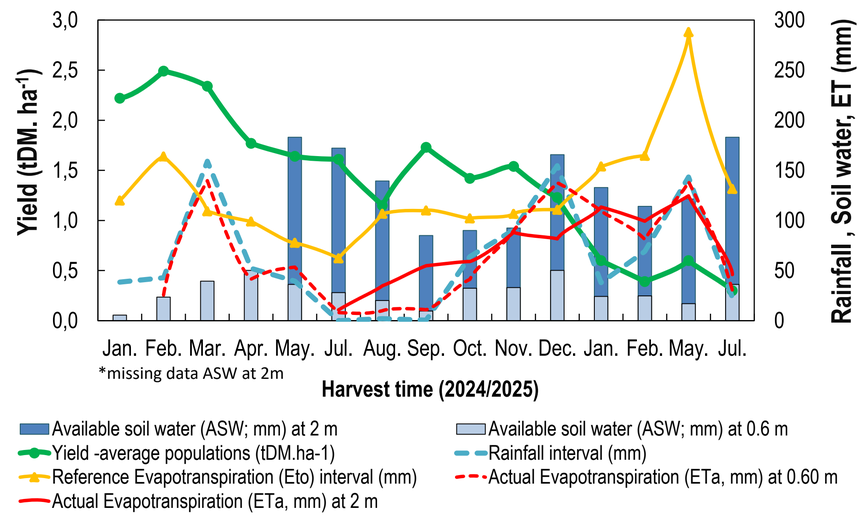


Fig.1. Data on the interval between harvests throughout the growth cycle of alfalfa populations from Jan. 2024 (harvest 1) to July 2025 (harvest 15) under natural conditions at INTA Santiago del Estero, Argentina.

Alfalfa yield showed a close relationship with **soil water availability (ASW), rainfall**, and **ETo**.
Periods with higher **ASW** and frequent **rainfall** events coincided with the highest **yields** particularly during the first harvests indicating sufficient water to meet crop demands.

During dry months, relatively stable yield resulted from water extraction from deeper soil layers.

After December, the pattern suggests that **WU** or **ETa** became limited by soil water deficits rather than by evaporative demand, coinciding with the period of highest maximum temperatures in the growing cycle (higher than 42°C).

Alfalfa's deep root system allows plants to maintain water uptake for extended periods as the upper layers dry out. However, when **ASW** approaches its lower limit (near the **Readily Available Water** or even the **Wilting Point**), water stress reduces photosynthesis, leaf area expansion, and regrowth, resulting in lower yields (as shown in Fig. 2).

Overall: Yield fluctuations across harvests reflected the dynamic interaction between rainfall inputs, soil water storage, and evapotranspiration, confirming that water availability was the main factor controlling production during the evaluation (Fig.3).

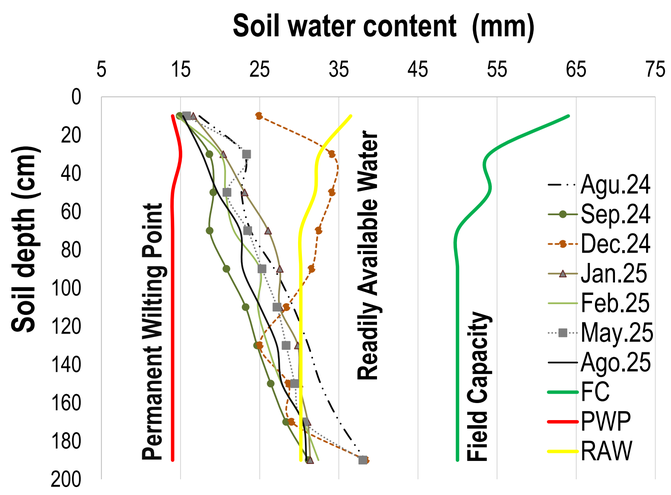


Fig.2. Soil water content up to 2 m depth on different dates (2024/25)

Fig.3. View of the trial at different times during the growth periods under natural conditions at INTA, Santiago del Estero, Argentina.

Yield and plant density

Harvest, Pop, and Interaction were all significant (p<0.001).This indicates that yield and density varied strongly across harvests, between Pop and that each Pop responded differently over time.

Despite the declining yield levels over time, Pop **PS2 and PS1 remained** the most productive on average, **Sal** showed intermediate performance, and **GB** was the least productive (Fig.3 , Table1).

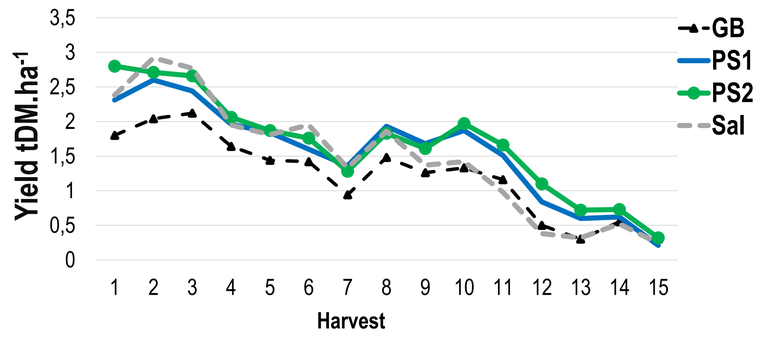


Fig.3. Yield by harvest per population under natural conditions at INTA, Santiago del Estero, Argentina.

Table 1. Mean yield and density per population among harvests.

Population	t DM.ha^{-1}	plant.m^{-2}
PS2	1,67 a	65 a
PS1	1,56 ab	62 ab
Sal	1,48 b	56 b
GB	1,22 c	48 c

Plant density and survival

PS2 and PS1 maintained the highest number of plants, retaining 42% of their initial stand (p<0.05). **GB** displayed intermediate performance, whereas **Sal** suffered the greatest decline, maintaining only about one-fourth of its initial stand (Table 2).

Table 2. Survival and density per population at the 15th harvest.

Population	% survival	plant.m^{-2}
PS2	42,03 ab	38 a
PS1	42,68 a	34 a
Sal	24,13 c	19 b
GB	33,36 b	26 b

Water use efficiency

The estimated WUE values (without statistical analysis) indicated that **PS1** and **PS2** had higher efficiency than GB and Sal (Table 3).

Table 3. Water use efficiency per population.

	PS1	PS2	Sal	GB
WUE (KgDM.mm^{-1})*	16,15	15,29	12,68	11,59

*for period Aug.24/Jul.25; total pp 586mm

Conclusion

Under the experimental conditions described, plants were subjected to moderate to severe water stress, which led to reduced water uptake, lower yield, and decreased water use efficiency.

The **PS1 and PS2** outperformed **GB**, likely due to their improved adaptation to soil water deficit conditions achieved through previous selection. Consequently, both will progress to the next phase of INTA's alfalfa breeding program.

(a) Annicchiarico et al., 2022 https://doi.org/10.1002/tpg2.20264