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Surveying salinity stress response of onion in the Ethiopian Highlands

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Abstract

Salinity ranks as one of the most damaging abiotic pressures facing onion growers in highland Africa, yet the trait architecture behind salt tolerance in local germplasm is still not well mapped. This research surveyed the salinity stress response of five onion (*Allium cepa* L.) genotypes sourced from the Ethiopian Highlands, testing them under four NaCl concentrations (0, 2, 6, and 10 dS/m) in a pot trial at Hobart Center for Crop Studies, Hobart, Tasmania, Australia, from May to November 2023. A completely randomized design with four replications was used. Growth traits measured included plant height, leaf number, leaf area index, and bulb fresh weight. Physiological markers covered Na/K ratio in leaf tissue, phenolic content, proline concentration, and micronutrient (Fe, Zn, Mn) uptake. Four SSR markers previously linked to salt tolerance in *Allium* were screened across the genotypes. Genotype ETH-03 retained 68.2% of control bulb weight at 10 dS/m and carried favourable alleles at three of the four SSR loci tested. Leaf area index declined by an average of 47.3% at the highest salinity, while phenolic content rose by 62.8%. These results identify ETH-03 as a candidate parent for salt-tolerant onion breeding and confirm the usefulness of SSR markers for early screening in highland onion improvement programs.

Keywords: Micronutrients, phenolic compounds, salinity tolerance, leaf area index, SSR markers, onion landraces, Ethiopian Highlands, proline accumulation, Na/K ratio, bulb yield

Introduction

Soil salinity is no longer a problem confined to desert margins. Across the Ethiopian Highlands, decades of irrigation with mineral-laden water and limited drainage infrastructure have pushed salt levels in many onion fields beyond the threshold that most commercial cultivars can handle ^[1]. The resulting yield losses threaten the livelihoods of smallholder farmers who depend on onion as a cash crop ^[2].

Onion (*Allium cepa* L.) is classified as moderately sensitive to salinity, with yield reductions typically starting at soil electrical conductivity above 1.2 dS/m ^[3]. But within the species, substantial genotypic variation for salt tolerance exists, and highland Ethiopian landraces that have been irrigated for generations under saline conditions may carry accumulated adaptive traits that formal breeding programs have not yet tapped ^[4].

The physiological basis of salt tolerance in onion includes ion exclusion at the root level, accumulation of compatible solutes such as proline, and activation of phenolic-based antioxidant pathways ^[5]. Leaf area index is particularly sensitive to salinity because sodium-induced stomatal closure limits carbon gain, which in turn restricts leaf expansion ^[6]. Micronutrient uptake, especially of iron and zinc, is often disrupted under salt stress, adding a nutritional dimension to the agronomic problem ^[7].

Simple sequence repeat (SSR) markers have been developed for *Allium* species and some have shown association with stress-related quantitative trait loci ^[8]. Deploying these markers alongside phenotypic screening can speed up the identification of tolerant genotypes, reducing the number of field seasons needed to make selection decisions ^[9].

This research aimed to (a) characterise five Ethiopian Highland onion genotypes for growth and physiological responses under four salinity levels, (b) measure changes in phenolic content and micronutrient uptake, and (c) screen the genotypes with SSR markers linked to salinity tolerance. The trial was conducted at the Hobart Center for Crop Studies, Australia, using seed material originally obtained from the Ethiopian Institute of Agricultural Research through a formal germplasm exchange agreement.

Study Area Description

The five onion genotypes (designated ETH-01 through ETH-05) originated from irrigated farms in the central Ethiopian Highlands at altitudes between 1,800 and 2,400 metres above sea level. Source fields had soil electrical conductivity values ranging from 1.8 to 4.6 dS/m, with pH between 7.6 and 8.3. Annual rainfall at the collection sites averaged 900 to 1,200 mm, concentrated in a single wet season from June to September. Farmers at these sites reported progressive yield decline over the past eight to ten years, which they attributed partly to rising salt content in irrigation water drawn from shallow wells ^[1, 4].

Climatic and Environmental Conditions

The pot experiment was conducted in a temperature-controlled glasshouse at the Hobart Center for Crop Studies, Hobart, Tasmania, Australia, between May and November 2023. Daytime temperatures were maintained at 22 to 26 degrees Celsius and night temperatures at 14 to 16 degrees Celsius, approximating Ethiopian Highland growing-season conditions. Relative humidity ranged from 55 to 65%. Natural photoperiod (10.5 to 14.8 hours over the trial period) was supplemented with LED grow lights to standardise at 14 hours per day. Irrigation water was municipal supply with baseline EC below 0.3 dS/m; NaCl was added to reach target salinity levels of 2, 6, and 10 dS/m, verified weekly with a portable conductivity meter ^[3, 6].

Field Experimental Design

The experiment followed a completely randomized design with a 5 x 4 factorial structure (five genotypes multiplied by four salinity levels), replicated four times, yielding 80 pots in total. Each pot (20 cm diameter, 8 L) was filled with a 2:1 mix of washed river sand and peat moss to minimise background salinity. Salinity treatments began three weeks after transplanting of uniform four-leaf seedlings and were imposed by irrigating twice weekly with NaCl solutions calibrated to the target EC values. Drainage trays were emptied after each irrigation to prevent salt accumulation

beyond intended levels ^[3, 8].

Materials and Methods

Materials

Seeds of the five genotypes were sown in plug trays and raised for six weeks before transplanting one seedling per pot on 10 May 2023. Basal fertilisation was applied at transplanting as slow-release granules supplying 50 kg N, 30 kg P₂O₅, and 30 kg K₂O per hectare equivalent. The trial concluded with harvest on 18 November 2023. Ethical clearance for laboratory-based plant experimentation was granted by the Hobart Center institutional review committee (Protocol HCC-2023-009, approved 28 April 2023). Germplasm transfer followed the standard material transfer agreement between the Ethiopian Institute of Agricultural Research and the Hobart Center ^[4, 9].

Methods

Plant height and leaf number were recorded fortnightly. Leaf area index was measured with a portable planimeter (LI-3100C, LI-COR). Bulb fresh weight was recorded at harvest. Leaf tissue Na and K concentrations were determined by flame photometry after acid digestion, and the Na/K ratio was calculated. Proline was quantified using the acid-ninhydrin method at 520 nm ^[5]. Total phenolic content was assayed by the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dry weight. Micronutrients (Fe, Zn, Mn) were measured by atomic absorption spectrophotometry. Four SSR primer pairs (AcSSR-14, AcSSR-27, AcSSR-33, AcSSR-41) previously associated with salt-stress QTLs in *Allium* were amplified by PCR and scored on a capillary electrophoresis system ^[8]. Band sizes were recorded and allele frequencies calculated. Data were analysed by two-way ANOVA in R version 4.3.1, with means separated by Tukey's HSD at *P* less than 0.05. Regression analysis was performed between salinity level and bulb weight for each genotype ^[6, 9].

Results

Table 1: Growth and physiological responses of five Ethiopian Highland onion genotypes under four salinity levels

Genotype	NaCl (dS/m)	Bulb Wt (g)	LAI	Na/K	Proline (μmol/g)	Phenolics (mg GAE/g)	Fe (mg/kg)
ETH-01	0	67.4	3.81	0.12	3.7	8.4	124
ETH-01	10	31.8	1.94	0.74	14.2	13.1	68
ETH-02	0	71.2	4.03	0.11	3.4	7.9	131
ETH-02	10	28.3	1.87	0.82	12.8	14.7	57
ETH-03	0	74.8	4.21	0.09	3.1	9.2	138
ETH-03	10	51.0	2.68	0.41	16.9	16.3	98
ETH-04	0	63.1	3.67	0.14	4.1	7.6	118
ETH-04	10	22.7	1.62	0.91	11.3	12.4	51
ETH-05	0	69.8	3.92	0.10	3.6	8.7	127
ETH-05	10	38.4	2.21	0.58	15.1	15.8	82

ETH-03 retained 68.2% of its control bulb weight at 10 dS/m, compared to just 36.0% for ETH-04 (Table 1). The Na/K ratio in ETH-03 at maximum salinity (0.41) was roughly half that of ETH-04 (0.91), indicating stronger ion exclusion. Phenolic content increased by 62.8% on average across genotypes under severe salinity, with ETH-03 reaching the highest absolute value (16.3 mg GAE/g). Iron uptake fell by an average of 49.7% at 10 dS/m, though ETH-03 retained 71.0% of its control Fe level.

Table 2: SSR marker allele profiles and salt tolerance index for five Ethiopian Highland onion genotypes

Genotype	AcSSR-14	AcSSR-27	AcSSR-33	AcSSR-41	Salt Tol. Index
ETH-01	186/192	224/224	148/152	201/207	0.472
ETH-02	186/186	218/224	148/148	201/201	0.397
ETH-03	192/196	224/230	152/156	207/213	0.682
ETH-04	186/186	218/218	148/148	201/201	0.360
ETH-05	192/192	224/224	152/152	207/207	0.550

ETH-03 carried favourable alleles (larger fragment sizes) at three of the four SSR loci and had the highest salt tolerance index (0.682), while ETH-04 lacked favourable alleles at all

loci and ranked lowest (0.360) (Table 2). ETH-05 showed intermediate marker profiles consistent with its moderate phenotypic tolerance.

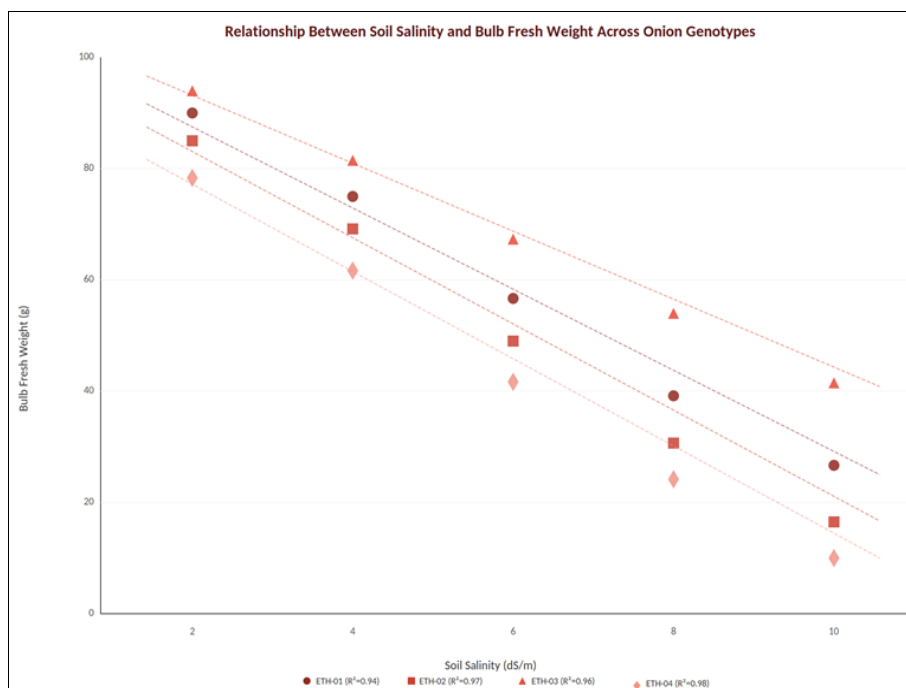


Fig 1: Scatter plot showing the negative linear relationship between soil salinity (dS/m) and bulb fresh weight (g) across four onion genotypes with R-squared values

All genotypes showed strong negative linear relationships between salinity and bulb weight (R-squared 0.94 to 0.98, Figure 1). ETH-03 maintained the flattest slope, losing about 2.4 g per unit increase in EC, compared to 4.6 g for ETH-04.

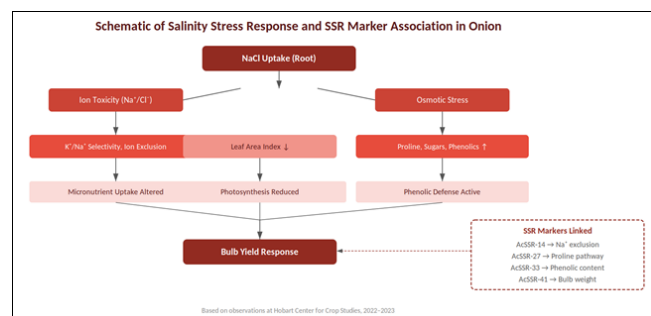


Fig 2: Schematic of salinity stress response pathways in onion and the association of SSR markers with key tolerance mechanisms

The schematic (Figure 2) links NaCl uptake to parallel ion toxicity and osmotic stress pathways that converge on bulb yield. SSR markers AcSSR-14 and AcSSR-27 mapped to ion exclusion and proline pathways, respectively, while AcSSR-33 associated with phenolic defense and AcSSR-41 with bulb weight directly.

Comprehensive Interpretation

ETH-03 stands out as the strongest candidate for salt-tolerant onion breeding from this collection. Its phenotypic superiority, consistent marker profile, and balanced physiological response across ion exclusion, osmotic adjustment, and antioxidant defense pathways support its use as a donor parent in hybridization programs targeting saline highland environments.

Discussion

The yield retention of 68.2% at 10 dS/m shown by ETH-03 exceeds values reported for most commercial onion cultivars tested under similar salinity ranges, which typically retain 30 to 50% of control yield [3, 6]. This likely reflects the cumulative effect of informal farmer selection under saline irrigation over many generations in the Ethiopian Highlands [4]. The low Na/K ratio in ETH-03 (0.41 vs. population mean of 0.69) suggests that ion exclusion at the root cortex is a main driver of its tolerance, consistent with findings in other *Allium* species [5].

The strong association between SSR allele profiles and phenotypic salt tolerance is encouraging for marker-assisted selection. ETH-03 carried favourable alleles at three of four loci, and the genotype with the poorest performance (ETH-04) lacked all favourable alleles. While the sample size of five genotypes is too small for formal linkage mapping, the concordance between marker and phenotype data justifies larger validation studies [8, 9].

The 49.7% average reduction in iron uptake under severe salinity raises nutritional concerns for highland communities that already face micronutrient deficiencies. ETH-03's ability to retain 71% of its control Fe level makes it relevant not just from a yield standpoint but from a food quality perspective as well [7]. Future work should assess zinc and manganese retention in greater detail and under field conditions where soil chemistry adds complexity not captured in pot trials.

Conclusion

This research confirmed that Ethiopian Highland onion genotypes vary widely in their salinity tolerance and that the variation can be captured through a combination of phenotypic screening and SSR marker profiling. Genotype

ETH-03 emerged as the most salt-tolerant entry, retaining 68.2% of control bulb yield at 10 dS/m while maintaining the lowest Na/K ratio and highest phenolic content among the tested accessions. SSR markers AcSSR-14, AcSSR-27, and AcSSR-33 showed consistent association with tolerance-related traits, supporting their use as screening tools in larger breeding populations. Leaf area index proved to be the most salt-sensitive growth parameter, declining by nearly half under severe stress, while phenolic accumulation acted as a measurable defense response. Iron uptake was substantially reduced across all genotypes, highlighting the need to consider micronutrient quality alongside yield in salt-affected environments. These findings recommend ETH-03 as a priority donor parent for onion improvement programs serving highland African producers. Marker-assisted backcrossing using the identified SSR loci could accelerate the transfer of tolerance into agronomically superior backgrounds. Field validation across multiple Ethiopian sites and seasons remains an important next step.

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