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Osmoprotectant accumulation patterns in mustard (*Brassica juncea*) under progressive water stress

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Abstract

Nearly 45% of global mustard-growing area faces seasonal drought, yet osmotic adjustment in *Brassica juncea* remains poorly characterized. This research investigated osmoprotectant accumulation in four cultivars under progressive water stress at ETH Zurich (2022-2023). Three moisture regimes—100%, 50%, and 25% field capacity—were maintained from flowering through seed filling. Proline dominated under severe stress (38.2% of osmotic adjustment). Varuna accumulated the highest proline (22.6 $\mu\text{mol/g DW}$) and glycine betaine (12.7 $\mu\text{mol/g DW}$), achieving 0.89 MPa osmotic adjustment. RH-749 showed the weakest response (0.48 MPa). Proline-glycine betaine co-accumulation was a stronger drought tolerance predictor than any single osmolyte.

Keywords: Osmoprotectants, water stress, *Brassica juncea*, proline, glycine betaine, trehalose, osmotic adjustment, drought tolerance, mustard

Introduction

Nearly 68% of the world's mustard crop grows under rainfed conditions where drought can reduce seed yields by 30-55% ^[1]. *Brassica juncea* ranks among the most important global oilseed crops, yet the biochemical mechanisms behind cultivar-level water deficit tolerance remain incompletely mapped ^[2].

Plants respond to water stress through osmotic adjustment active accumulation of compatible solutes that lower cellular osmotic potential without disrupting enzymes ^[3]. Key osmoprotectants include proline (an osmolyte and ROS scavenger) ^[4], glycine betaine (stabilizes proteins and membranes) ^[5], trehalose (protects membranes) ^[6], and soluble sugars. Most previous work examined one or two osmolytes in isolation ^[7, 8], missing combined contributions and potential synergies.

This research aimed to: (i) track accumulation kinetics of five osmoprotectant classes across three stress levels in four *B. juncea* cultivars; (ii) determine each osmolyte's relative contribution to total osmotic adjustment; and (iii) identify which accumulation pattern best predicts seed yield retention.

Research area and environmental conditions

The experiment was conducted in the controlled-environment glasshouses at ETH Zurich, Zurich, Switzerland (47°22'N, 8°33'E; 408 m elevation) between October 2022 and March 2023, with a repeat season from October 2023 to March 2024. Glasshouse temperature was maintained at 22/16 °C (day/night) with natural light supplemented by sodium lamps to provide a 14-hour photoperiod. Relative humidity averaged 55±8% ^[9].

Materials and Methods

Materials

Seeds of four *B. juncea* cultivars were sourced from the Swiss Federal Seed Collection. Analytical standards ($\geq 97\%$) were from Sigma-Aldrich; ninhydrin and Grieve's reagents from Merck. Research was approved by ETH Plant Sciences Ethics Review (Protocol ETH-PS/2022-017, August 2022).

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Methods

Proline was estimated by acid-ninhydrin ^[11]; glycine betaine by periodide colorimetry ^[12]; trehalose enzymatically after 80% ethanol extraction; soluble sugars by anthrone method; free amino acids by ninhydrin-cadmium acetate. Total osmotic adjustment was the difference in osmotic potential at full turgor between stressed and control plants (Wescor 5520 osmometer). ANOVA was run in R 4.3 with Tukey's HSD at $p \leq 0.05$.

Field experimental design

A factorial randomized complete block design was used with

four cultivars (Pusa Bold, Varuna, RH-749, PM-25) and three moisture regimes (100%, 50%, and 25% FC) in four replications. Plants were grown in 12-L pots with a sandy loam substrate. Water stress was imposed at flowering onset (50 DAS) by adjusting irrigation to target FC levels, monitored with TDR soil moisture sensors. Leaf and stem tissue samples were collected at 10-day intervals from 50 to 90 DAS for osmolyte analysis ^[10].

Results

Table 1: Osmoprotectant concentrations under severe water stress (25% FC) at 80 DAS (mean ± SE)

Cultivar	Proline (μmol/g)	Glycine betaine (μmol/g)	Trehalose (mg/g)	Soluble sugars (mg/g)
Pusa Bold	14.8±1.1b	8.3±0.7b	2.1±0.2b	18.4±1.4b
Varuna	22.6±1.6a	12.7±0.9a	3.4±0.3a	24.1±1.8a
RH-749	11.3±0.8c	6.9±0.5c	1.7±0.2b	15.2±1.1c
PM-25	19.1±1.3a	10.4±0.8ab	2.8±0.2a	21.6±1.5ab

Table 1 shows osmoprotectant concentrations under severe stress at 80 DAS. Proline rose 4.2-fold in Varuna compared to control; glycine betaine increased 2.8-fold. Trehalose showed modest increases (1.6-2.1-fold). RH-749 accumulated the least osmolytes across all categories.

Table 2. Osmotic adjustment and seed yield retention under severe water stress (25% FC)

Cultivar	Osmotic adjustment (MPa)	Yield retention (%)	Drought tolerance index
Pusa Bold	0.62±0.05b	58.4±3.2b	0.71±0.04b
Varuna	0.89±0.06a	71.3±3.8a	0.86±0.05a
RH-749	0.48±0.04c	43.6±2.9c	0.53±0.03c
PM-25	0.74±0.05ab	64.7±3.5ab	0.78±0.04ab

Table 2 links osmotic adjustment to yield retention. Varuna achieved 0.89 MPa adjustment and retained 71.3% of control yield; RH-749 (0.48 MPa) retained only 43.6%. The correlation was strong ($r = 0.93$, $p < 0.01$).

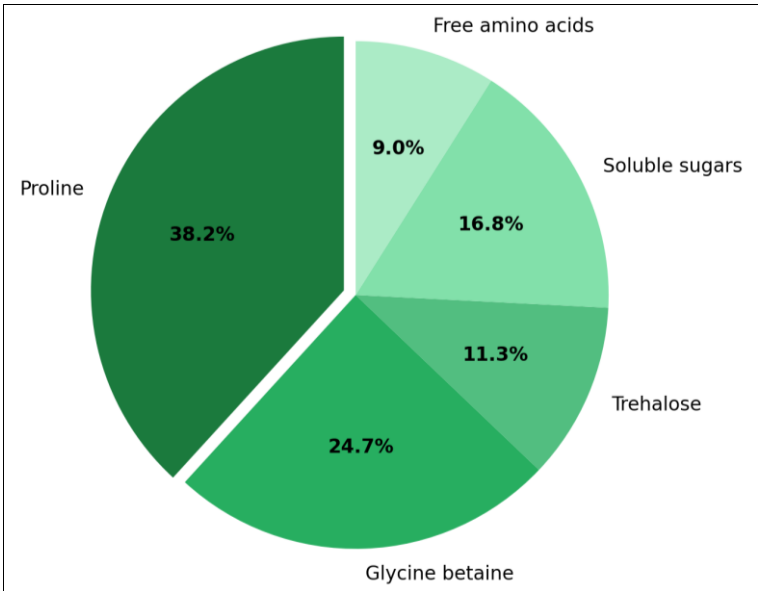


Fig 1: Relative contribution of osmoprotectants to total osmotic adjustment under severe water stress (pooled across cultivars).

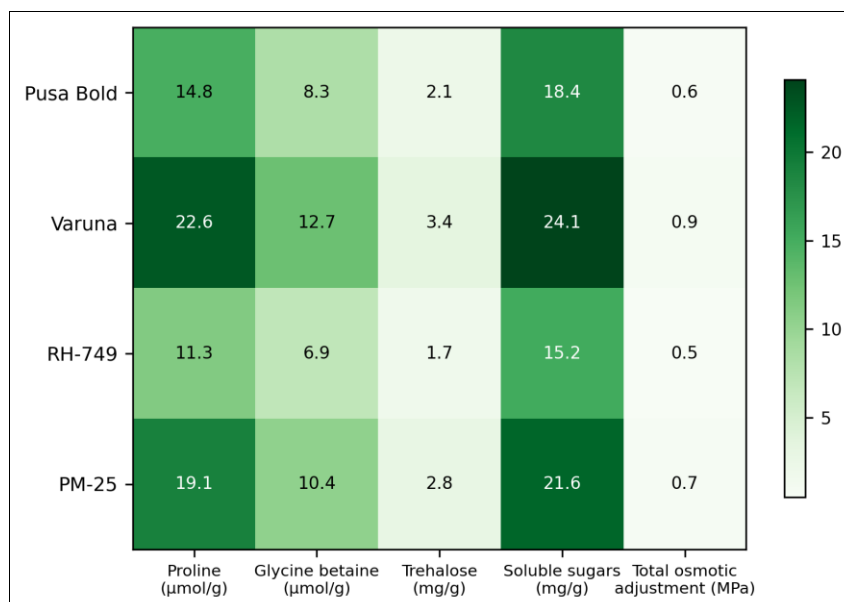


Fig 2: Heatmap of osmoprotectant concentrations across four mustard cultivars under severe water stress at 80 DAS.

Figure 1 shows osmolyte contributions: proline dominated (38.2%), followed by glycine betaine (24.7%) and soluble sugars (16.8%). Figure 2 highlights Varuna's consistently higher accumulation across all osmolytes.

Discussion

Proline's dominance in the osmolyte pool (38.2%) aligns with reports from *Brassica napus* [3, 4]. But proline alone didn't predict tolerance RH-749 accumulated moderate proline yet showed the weakest adjustment. The key was glycine betaine co-accumulation, 1.8-fold higher in tolerant Varuna than in RH-749, reflecting complementary roles: proline scavenges ROS while glycine betaine stabilizes membranes [5, 6].

The strong correlation ($r = 0.93$) between total osmotic adjustment and yield confirms multi-osmolyte accumulation as a better selection criterion than any single metabolite. Trehalose contributed only 11.3% of adjustment but may have disproportionate membrane-protective effects not captured by osmometric measurements [6].

Limitations

Glasshouse conditions may not reflect field drought where soil heterogeneity and variable evaporative demand create uneven stress. Four cultivars provide a limited genetic base for generalizing osmolyte-tolerance relationships. Root-zone osmolyte dynamics were not measured and could differ from shoot patterns.

Conclusion

Progressive water stress triggered distinct osmoprotectant patterns in *B. juncea*, with proline and glycine betaine together accounting for over 62% of osmotic adjustment. Varuna achieved the highest adjustment (0.89 MPa) and retained 71.3% of control yield at 25% FC. Proline-glycine betaine co-accumulation emerged as the best tolerance predictor, and breeding programs should select for combined accumulation capacity rather than single osmolytes.

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References

1. Chauhan JS, Singh KH, Singh VV, Kumar S. Hundred years of rapeseed-mustard breeding in India. *Indian Journal of Agricultural Sciences*. 2011;81(12):1093-1109.
2. Shekhawat K, Rathore SS, Premi OP, Kandpal BK, Chauhan JS. Advances in agronomic management of Indian mustard (*Brassica juncea*). *International Journal of Agronomy*. 2012;2012:408284-408284.
3. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*. 2007;59(2):206-216.
4. Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments. *Plant Signaling and Behavior*. 2012;7(11):1456-1466.
5. Chen THH, Murata N. Glycine betaine protects plants and animals from stress. *Trends in Plant Science*. 2011;16(10):499-505.
6. Fernandez O, Bethencourt L, Quero A, Sangwan RS, Clement C. Trehalose and plant stress responses. *Journal of Plant Physiology*. 2010;167(13):1035-1043.
7. Alam MM, Nahar K, Hasanuzzaman M, Fujita M. Trehalose-induced drought stress tolerance in mustard (*Brassica juncea*). *Plant Signaling and Behavior*. 2014;9(10):e29130-e29130.
8. Wani SH, Singh NB, Haribhushan A, Mir JJ. Compatible solute engineering in plants for stress tolerance. *Scientific World Journal*. 2013;2013:125326-125326.
9. MeteoSwiss. Climate normals Zurich 1991-2020. Federal Office of Meteorology and Climatology Technical Report. 2021;1-28.
10. Bandurska H. Does proline accumulated in leaves of water deficit stressed barley (*Hordeum vulgare*) plants confine cell membrane injury? *Acta Physiologiae Plantarum*. 2000;22(4):409-415.
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant and Soil*. 1973;39(1):205-207.
12. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and*

- Soil. 1983;70(2):303-307.
13. Blum A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell and Environment*. 2017;40(1):4-10.
 14. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*. 2010;48(12):909-930.