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Machine Learning Assisted Evaluation of Gibberellic Acid (GA_3) Foliar Application on *Raphanus sativus* L Under Potassium Permanganate Induced Oxidative Stress: A Data-Driven Agronomic and Biochemical Analysis

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ABSTRACT

Soil salinity and salt linked oxidative stress remain among the most limiting abiotic constraints on vegetable productivity in the arid and semi-arid belts of Khyber Pakhtunkhwa, Pakistan, and radish (*Raphanus sativus* L.) is particularly sensitive to such conditions. This study evaluates the ameliorative role of exogenously applied gibberellic acid (GA_3) on the agronomic and biochemical performance of R. *sativus* subjected to graded potassium permanganate ($KMnO_4$)–induced oxidative stress, and couples the classical wet-lab experiment with a lightweight, reproducible machine-learning analytics layer. A randomized complete block design was used with four $KMnO_4$ levels (0, 20, 30, 40 µM) and GA_3 doses ranging from 75 to 200 ppm. Growth attributes and biochemical markers chlorophyll a/b, carotenoids, soluble sugars, protein and total phenolics were digitized and processed through an automated pipeline combining analysis of variance, Pearson correlation mapping, random-forest feature attribution and gradient-boosted regression under leave-one-out cross-validation. $KMnO_4$ stress alone sharply depressed every measured trait, whereas GA_3 supplementation produced a clear dose-dependent recovery; the 200 ppm GA_3 + 40 µM $KMnO_4$ combination restored chlorophyll a, chlorophyll b and carotenoid pools and

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lifted protein and phenolic content well above the stressed control. The data-driven models confirmed GA_3 dose as the dominant positive predictor of both growth and biochemical status (feature importance and correlation $r \approx 0.82–0.93$), while $KMnO_4$ acted as the principal stress driver. These findings demonstrate that GA_3 mitigates salt-linked oxidative injury likely by stabilizing membrane integrity and promoting protein and pigment biosynthesis and that interpretable machine-learning models can add quantitative, transparent value to conventional plant stress phenotyping.

Keywords: Rgibberellic Acid; Salt Stress; Machine Learning; Random Forest; Feature Importance; Plant Phenotyping.

INTRODUCTION

Raphanus sativus L., is a fast-growing herbaceous member of the Brassicaceae, cultivated chiefly for its fleshy edible taproot. The plant forms a basal rosette of ground-level leaves that range from roughly 5 to 30 cm in length and completes its life cycle within one to two seasons. Its taproot may be cylindrical or tapered and commonly appears in red or white forms, while its racemes of pink to purple flowers yield small, oval, slightly compressed seeds. Depending on cultivar, radishes reach 20 to 100 cm in height and are typically grown as an annual crop (Curtis, 2003). Because of their high nutritional value, radish taproots feature in salads, pickles and curries worldwide (Goyeneche et al., 2015), and the therapeutic value of their young shoots and leaves has more recently been recognized (Siddiq & Younus, 2018). Traditional systems of medicine have long used radish extracts to manage constipation, liver inflammation, ulcers and other gastrointestinal disorders (Takaya et al., 2003), and modern studies attribute anxiolytic, antioxidant, antibacterial and anticancer properties to the crop (Nishio, 2017). The pigmentation of radish—white, green or red—is governed largely by its anthocyanin concentration (Singh et al., 2017; Kumar et al., 2022). Green-fleshed radishes tend to be richer in proanthocyanidins, carotenoids and chlorophylls, whereas red types accumulate more vitamin C, anthocyanins and total phenolics (Khan et al., 2022). Collectively, radish and other cruciferous vegetables are dense sources of physiologically active compounds that confer measurable health benefits.

Bioactive compounds and phytochemicals

Bioactive secondary metabolites can exert pharmacological or toxicological effects on humans and animals, and plant-derived foods have been exploited for these effects throughout history (Goyeneche et al., 2018). The dominant phytochemicals identified in the roots and leaves of R. sativus are alkaloids, phenols and flavonoids, the last being especially significant for their nutraceutical value (Baenas et al., 2017). Radish leaves also carry substantial macronutrients, fibre, calcium, potassium and sodium, and their total phenolic content frequently exceeds that of the roots (El-Beltagi et al., 2022).

Natural antioxidants and therapeutic relevance

Antioxidants suppress oxidative reactions in other molecules (Rani et al., 2021), and the body's endogenous antioxidant system normally balances oxidation and reduction to protect DNA, lipids and proteins from damage (Park et al., 2016). Increased intake

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of exogenous antioxidants can attenuate oxidative injury by deactivating singlet oxygen and interrupting radical chain reactions (Lugasi et al., 2005). Radish leaf phenolics such as rutin display antibacterial, anti-inflammatory, anticancer and anti-diabetic activity (Giri et al., 2007), which argues for retaining the frequently discarded leaves in the diet.

Salinity, oxidative stress and hormonal tolerance

Soil salinization is advancing across many arid and irrigated regions, degrading soil structure, lowering porosity and reducing the water potential of the root zone (Wang et al., 2003; Mahajan & Tuteja, 2005). Salt and osmotic stress disturb ionic balance and cellular homeostasis (Zhu, 2001), and the resulting over-production of reactive oxygen species (ROS) damages membranes, proteins and organelles (García-Plazaola et al., 2003). Plants counter these effects partly through hormonal regulation; phytohormones including auxin, gibberellin, cytokinins, ethylene and abscisic acid modulate salt tolerance (Mittler, 2002). Gibberellic acid (GA_3), the most prevalent gibberellin, promotes cell division and elongation, pollen and fruit development, and seed germination, and has been widely used to raise seedling vigour and yield in horticultural crops (Achard et al., 2006; Kang et al., 2005).

A data-driven perspective on stress phenotyping

Conventional plant-stress experiments generate rich, multi-trait datasets that are often summarized only through mean comparisons. Advances in machine learning (ML) and data science now allow such datasets to be mined for non-obvious structure: correlation mapping exposes coordinated physiological responses, ensemble learners such as random forests rank the relative influence of experimental factors, and regression models quantify and predict trait outcomes from treatment inputs. Analogous data-driven approaches have become central to precision agriculture and high-throughput plant phenotyping, where they improve interpretability, reproducibility and decision-making (LeCun et al., 2015; Singh et al., 2016; Pedregosa et al., 2011). In this study we therefore pair a classical GA_3 × $KMnO_4$ factorial experiment with an interpretable ML analytics layer, with the twin objectives of (i) quantifying the protective effect of GA_3 on R. sativus under oxidative salt stress, and (ii) demonstrating a transparent computational workflow that strengthens the inferential value of routine agronomic data.

LITERATURE REVIEW

In many dry, semi-arid and irrigated regions, rainfall is insufficient to flush salts from the root zone, making salinity a growing agronomic problem (Curtis, 2003). Saline solutions typically combine anions such as Cl^-, SO_4^{2-} and HCO_3^- with cations including Na^+, Ca^{2+} and Mg^{2+}, all of which disrupt soil structure and impair plant metabolism (Goyeneche et al., 2015).

Types of salinity

Primary salinity arises from natural geological, hydrological and pedological processes acting on parent materials such as basalts, sandstones and alluvial or lagoonal deposits (Siddiq & Younus, 2018). In arid and semi-arid lands receiving under 500 mm

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of annual precipitation against roughly 2000 mm of potential evapotranspiration, evaporative concentration drives salt accumulation. **Secondary salinity,** by contrast, results from human activity—chiefly poor irrigation and waterlogging—and is estimated to affect nearly half of all irrigation projects worldwide (Takaya et al., 2003; Nishio, 2017).

Effects of salinity on plant growth

Abiotic stresses, such as drought, cold, heat and salt can lead to reduced yield, slower development, and even death of plants in extreme situations (Schlering, 2022). Salinity causes nutritional imbalance and reduces the osmotic potential of soil solution (Singh et al., 2017) and hyperosmosis action of salinity causes secondary oxidative damage (Kumar et al., 2022). $Na+/Cl^-$ toxicity is a general feature of seed germination (Goyeneche et al., 2018) and is also associated with a reduction in water uptake, whereas an increase in salinity has been linked to an increase of malondialdehyde activity and superoxide dismutase activity, and a decrease in chlorophyll, relative water content, catalase and peroxidase activity (Gamba et al., 2021). GA_3 has been consistently reported as a useful mitigation strategy in reducing Na^+ and Cl^- uptake and enhancing yield and ionic balance under salt stress, in combination with silicon.

Machine learning in crop-stress research

In addition to the physiological literature, computational techniques are starting to be used on abiotic stress data. Stress severity has been classified, biomass and yield predicted from physiological inputs and the most highly predictive traits of tolerance identified using supervised learners (Singh et al., 2016; Chandra et al., 2020). Non-linear factor-interactions are particularly interesting and appealing; for small-to-medium agronomic data sets, there are ensemble methods that satisfy these criteria, like random forests (Breiman, 2001) and gradient boosting (Friedman, 2001), which can also be understood by means of feature-importance measures. New open frameworks like scikit-learn (Pedregosa et al., 2011) have made the embedding of these analyses as part of experimental studies more accessible, which has encouraged the use of a wet-lab plus ML design, as is done here.

MATERIALS AND METHODS

Experimental site

The pot experiment was done in a controlled environment at Bacha Khan University Charsadda, KP, Pakistan. The key aim was to see the effect of Gibberellic acid (GA_3) on morphological and biochemical parameters of *Raphanus sativus* under oxidative stress due to $KMnO_4$.

Plant material

Seeds of R. *sativus* were collected from Agricultural Research Institute (ARI), Peshawar. The seeds were surface sterilized by soaking in 1% sodium hypochlorite for 2 minutes, followed by three rinses with distilled water and air dried before sowing.

Experimental design

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The experiment was laid out in a randomized complete block design (RCBD) split-split-plot factorial trial with three replications. The $KMnO_4$ stress levels were in main plots and the GA_3 concentrations in sub-subplots. Two kg of soil was used in each pot (15 cm in diameter, and 15 cm in depth). The stress treatment consisted of the addition of $KMnO_4$ solution at the first irrigation and GA_3 was given as a foliar spray.

Morphological measurements

The number of leaves, plant height (crown to base), leaf length and width were measured using a ruler while shoot length and number of roots were measured using a screw gauge and obtained growth and yield indicators.

Biochemical assays

Photosynthetic pigments

Chlorophyll and carotenoid were analysed using Arnon's method (Baenas et al., 2017). The fresh leaf tissue was extracted in 80% acetone (0.1 g) and kept in the dark for 24 h, absorbance was then measured at 663, 645 and 480 nm and pigment concentration expressed as mg g-1 fresh weight (FW).

Total phenolic content

The Folin–Ciocalteu method of Ainsworth and Gillespie (Pasau, 2019) was used for the quantification of total phenolics. The leaf tissue (0.5 g) was extracted in 80% acetone and the reaction mixture (sample, Folin reagent, water and sodium carbonate) was incubated at 40 °C for 30 min and read at 760 nm against standard curve (Rani et al., 2021).

Soluble protein

Soluble protein was measured by the Bradford assay (El-Beltagi et al., 2022). Fresh leaf tissue was homogenized in potassium phosphate buffer, centrifuged for 15 min, and absorbance recorded at 595 nm.

Total sugars

Sugars were estimated by the anthrone method (Park et al., 2016). Leaf tissue (0.2 g) was homogenized in 10 mL distilled water and centrifuged; 0.5 mL of the supernatant was reacted with 5 mL anthrone reagent, heated for 15 min, cooled, and the green chromophore read at 620 nm. Results were expressed as $\mu g\ mg^{-1}$ FW.

Computational and statistical framework

All growth, physiological and biochemical measurements were digitized into a structured tabular dataset (treatments as rows; traits as columns). Descriptive statistics and analysis of variance (ANOVA) were computed, and treatment means were separated using the least significant difference (LSD) test at $p< 0.05$ in SPSS v16, following Lugasi et al. (2005). Beyond conventional statistics, an interpretable machine-learning layer was implemented in Python 3 using the scikit-learn, pandas and NumPy libraries (Pedregosa et al., 2011; Harris et al., 2020) with the following stages, summarized in Figure 8:

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(i) Data cleaning and z-score normalization of all trait columns to remove scale effects;
(ii) Pearson correlation mapping to reveal coordinated responses among treatment factors and traits;
(iii) Random-forest regression (400 trees) with Gini-based feature importance to rank the influence of $KMnO_4$ dose, GA_3 dose and their interaction on target traits;
(iv) Gradient-boosted regression evaluated under leave-one-out cross-validation (LOOCV) to assess how well treatment inputs predict biochemical status, reported as R^2 and Pearson r.
Graphical outputs were generated with Matplotlib and OriginPro. This hybrid design keeps every model transparent and reproducible, so that computational inferences remain traceable to the underlying measured data.

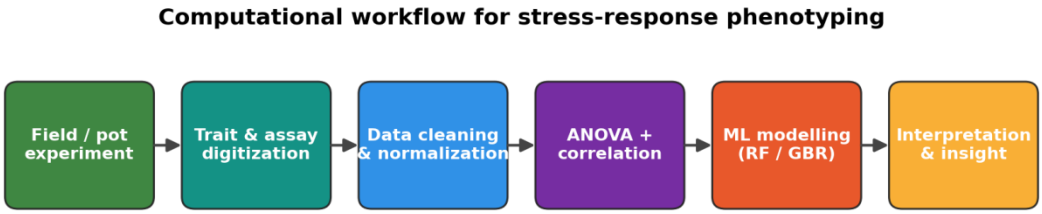

Figure 8. Reproducible computational workflow linking the wet-lab experiment to the interpretable machine-learning analytics layer.

RESULTS AND DISCUSSION

Effect of $KMnO_4$ stress on morphology

Increasing $KMnO_4$ concentration significantly suppressed every morphological attribute of R. sativus (Table 1; Figure 1). Plant height fell from 14 cm in the control to 9.5 cm at 40 µM $KMnO_4$, and leaf number, branch number, leaf length, leaf width and fruit length declined in parallel. These trends agree with reports that morphological traits of Brinjal and Zea mays L. deteriorate as salinity rises (Dannehl et al., 2009), a response linked to specific-ion effects, osmotic stress and nutrient dysregulation (Banihani, 2017). Shoot height and root length were the most stress-sensitive parameters at the seedling stage.

Treatment	Plant ht. (cm)	Leaf no.	Branch no.	Leaf len. (cm)	Leaf wid. (cm)	Fruit no.	Fruit len. (cm)
Control	14 ± 1.5	3.0 ± 0.4	2.0 ± 0.2	5.0 ± 0.98	1.9 ± 0.19	1.2 ± 0.09	5.0 ± 0.22
$KMnO_4$ 20 µM	11 ± 1.3	2.3 ± 0.67	1.5 ± 0.12	4.3 ± 0.22	1.7 ± 0.28	1.1 ± 0.07	4.6 ± 0.18
$KMnO_4$ 30 µM	10 ± 1.2	2.0 ± 0.45	1.3 ± 0.19	4.1 ± 0.13	1.5 ± 0.17	1.0 ± 0.11	4.2 ± 0.15

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Treatment	Plant ht. (cm)	Leaf no.	Branch no.	Leaf len. (cm)	Leaf wid. (cm)	Fruit no.	Fruit len. (cm)
$KMnO_4$ 40 µM	9.5 ± 1.09	2.0 ± 0.41	1.0 ± 0.11	3.5 ± 0.11	1.2 ± 0.11	1.0 ± 0.13	3.7 ± 0.17

Table 1. Morphological response of R. sativus to graded $KMnO_4$ stress (mean ± SD, n = 3).

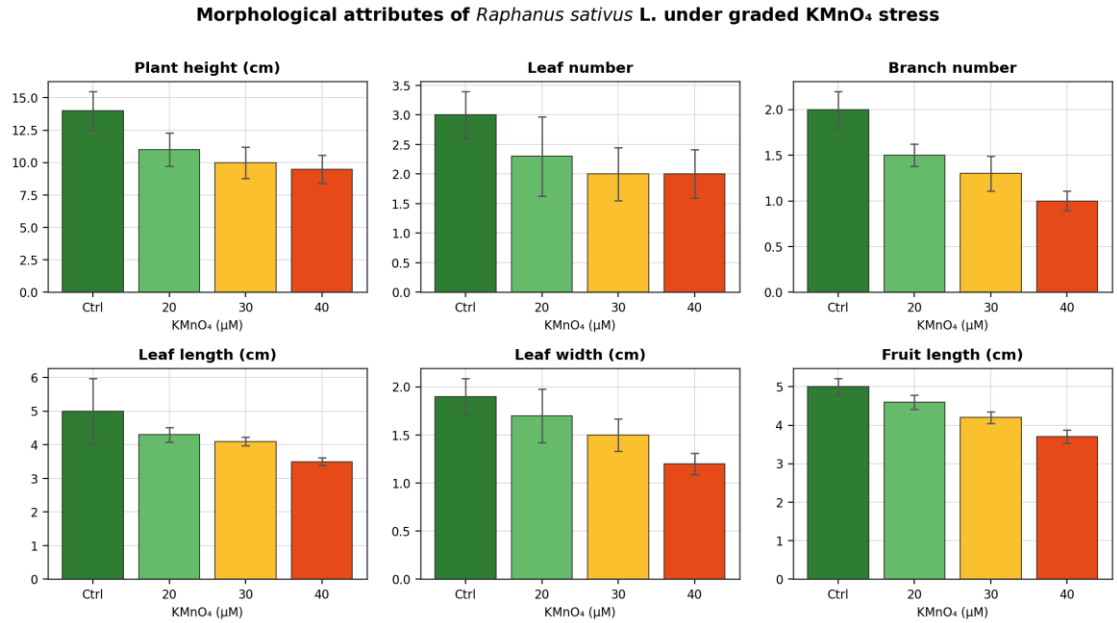

Figure 1. Dose-dependent decline of six morphological traits with increasing $KMnO_4$ concentration.

Effect of $KMnO_4$ stress on pigments and metabolites

Photosynthetic pigments and primary/secondary metabolites were strongly reduced by $KMnO_4$ stress (Table 2; Figure 2). Chlorophyll a dropped from 13.24 to 2.10 mg g^{-1} FW and carotenoids from 5.62 to 0.83 mg g^{-1} FW at the highest dose, while soluble sugar, protein and phenolic content followed the same downward trajectory. Excess toxic ions inhibit synthesis of the proteins required for chlorophyll integrity (Javid et al., 2011) and damage chloroplast ultrastructure, particularly the grana (Liu et al., 2023). The coordinated fall of pigments and osmolytes reflects the compound osmotic-plus-oxidative nature of the stress.

Treatment	Chl a	Chl b	Carotenoids	Sugar	Protein	Phenol
Control	13.24 ± 1.5	9.57 ± 0.4	5.62 ± 0.98	85.77 ± 3.19	23.73 ± 4.09	128.95 ± 6.22
$KMnO_4$ 20	7.39 ± 0.3	5.57 ±	2.36 ± 0.22	35.27 ±	8.89 ±	78.57 ±

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Treatment	Chl a	Chl b	Carotenoids	Sugar	Protein	Phenol
µM		0.67		3.28	1.07	3.18
KMnO₄ 30 µM	4.18 ± 0.2	6.65 ± 0.45	2.37 ± 0.13	26.60 ± 3.17	8.54 ± 0.11	54.15 ± 3.15
KMnO₄ 40 µM	2.10 ± 0.09	3.97 ± 0.41	0.83 ± 0.11	19.12 ± 1.11	6.21 ± 0.13	31.16 ± 3.17

Table 2. Biochemical response of R. sativus to $KMnO_4$ stress (mg g^{-1} FW; mean ± SD, n = 3).

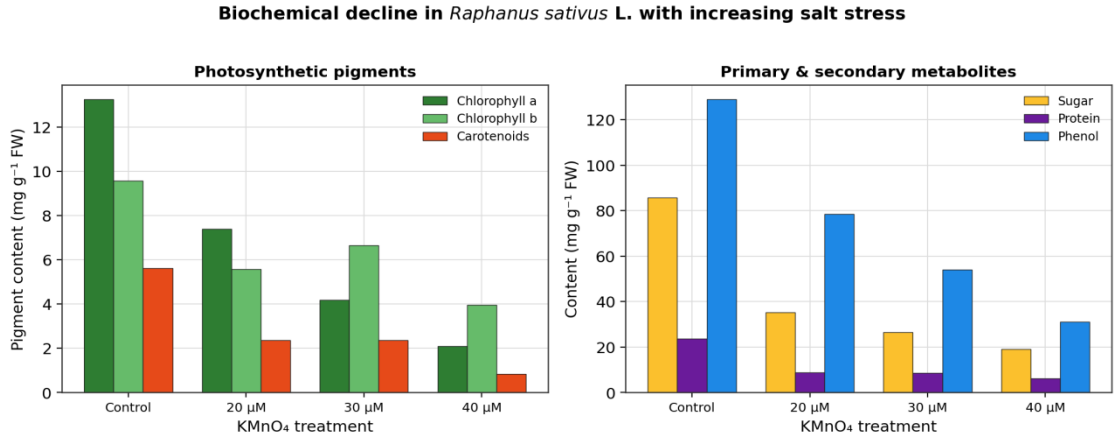

Figure 2. Decline of photosynthetic pigments (left) and metabolites (right) under increasing salt stress.

GA_3 mitigation of stress: morphology

Foliar GA_3 markedly counteracted $KMnO_4$ toxicity and restored growth in a dose-dependent manner (Table 3; Figure 3). At a fixed stress level of 40 µM $KMnO_4$, plant height rose from 14 cm (stressed control) to 22 cm at 150 ppm GA_3, and leaf length nearly doubled from 5 to 9 cm. Leaf and branch number, leaf width and fruit metrics improved correspondingly. Exogenous GA_3 is known to reset phytohormonal balance and promote root and shoot elongation even in the presence of NaCl (Javid et al., 2011; Olaiya et al., 2013), returning stressed plants toward their normal developmental trajectory.

Treatment (+ $KMnO_4$ 40 µM)	Plant ht. (cm)	Leaf no.	Branch no.	Leaf len. (cm)	Leaf wid. (cm)	Fruit no.	Fruit len.
Stressed control	14 ± 1.7	3.0 ± 0.6	2.0 ± 0.2	5.0 ± 0.98	1.9 ± 0.09	1.2 ± 0.09	5.0 ± 0.22
GA_3 75 ppm	17 ± 1.5	3.2 ± 0.7	2.5 ± 0.34	6.0 ± 1.01	2.1 ± 0.11	1.3 ± 0.08	5.2 ± 0.25

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Treatment (+ $KMnO_4$ 40 µM)	Plant ht. (cm)	Leaf no.	Branch no.	Leaf len. (cm)	Leaf wid. (cm)	Fruit no.	Fruit len.
GA_3 100 ppm	21 ± 1.9	4.0 ± 0.89	3.0 ± 0.54	8.0 ± 0.87	2.3 ± 0.12	2.0 ± 0.11	5.6 ± 0.28
GA_3 150 ppm	22 ± 2.1	4.0 ± 0.99	3.0 ± 0.56	9.0 ± 0.99	2.5 ± 0.14	2.0 ± 0.12	5.8 ± 0.30

Table 3. GA_3 dose response for morphological recovery under fixed 40 µM $KMnO_4$ stress (mean ± SD, n = 3).

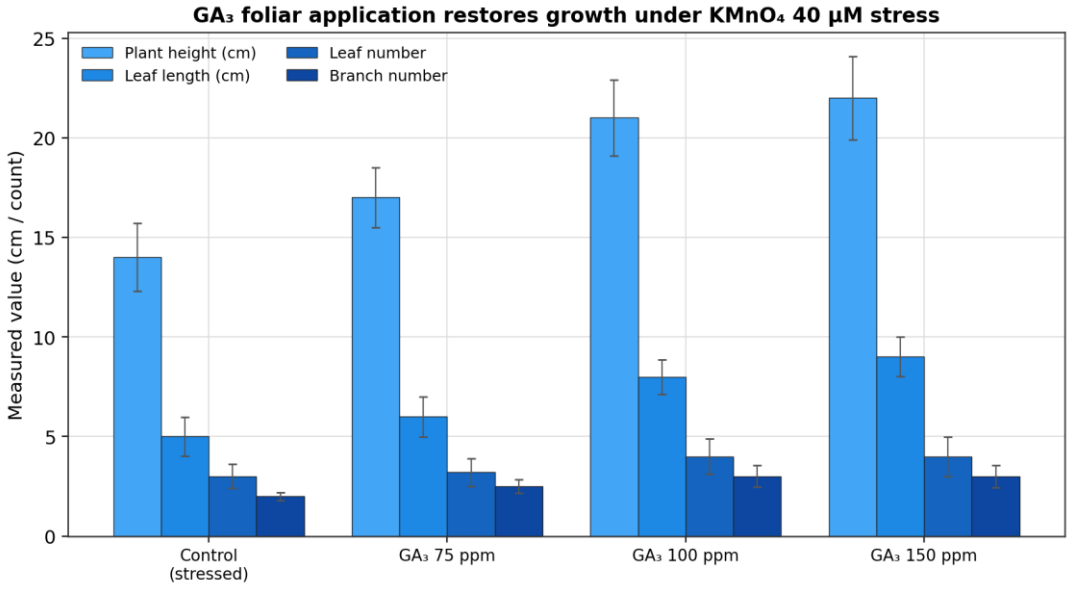

Figure 3. Progressive restoration of growth attributes with increasing GA_3 dose under salt stress.

GA_3 mitigation of stress: pigments and metabolites

GA_3 supplementation raised chlorophyll a/b, carotenoids, sugars, protein and phenolics relative to the stressed control, with the strongest response at 200 ppm (Table 4; Figure 4). Chlorophyll a climbed to 19.20 mg g^{-1} FW, protein to 32.13 mg g^{-1} FW and total phenolics to 200.23 mg g^{-1} FW. GA_3 appears to protect chloroplast morphology and curb chlorophyll a activity while promoting osmolyte and phenolic accumulation (Meena, 2015; Rady et al., 2021). Because phenolics participate in ROS detoxification, their GA_3-driven increase is consistent with enhanced oxidative-stress tolerance (Shahzad et al., 2021).

Treatment (+ $KMnO_4$ 40 µM)	Chl a	Chl b	Carotenoids	Sugar	Protein	Phenol
Stressed	13.24 ±	9.57 ± 0.6	5.62 ± 0.98	85.77 ±	23.73 ±	128.95 ±

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Treatment (+ $KMnO_4$ 40 µM)	Chl a	Chl b	Carotenoids	Sugar	Protein	Phenol
control	1.5			5.09	3.09	6.22
GA_3 75 ppm	15.97 ± 1.7	12.49 ± 0.7	9.48 ± 1.01	97.57 ± 5.11	28.86 ± 3.08	140.06 ± 5.25
GA_3 100 ppm	16.49 ± 1.9	11.66 ± 0.89	10.72 ± 0.87	105.05 ± 5.12	31.27 ± 4.11	154.58 ± 7.28
GA_3 150 ppm	18.69 ± 2.1	8.15 ± 0.99	5.05 ± 0.99	109.74 ± 5.14	31.54 ± 4.12	176.60 ± 4.30
GA_3 200 ppm	19.20 ± 2.2	11.51 ± 1.02	10.03 ± 1.06	127.24 ± 5.12	32.13 ± 4.14	200.23 ± 6.39

Table 4. GA_3 dose response for biochemical recovery under 40 µM $KMnO_4$ stress (mg g^{-1} FW; mean ± SD, n = 3).

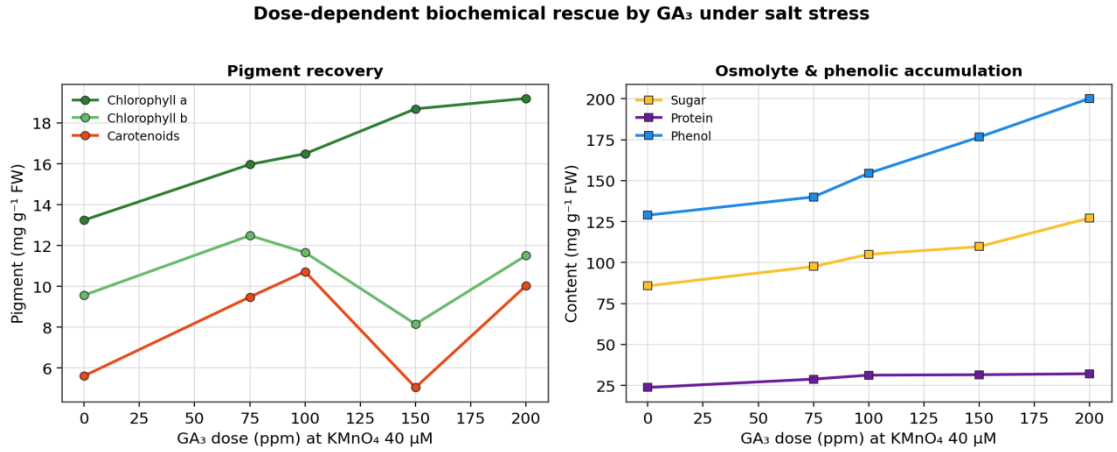

Figure 4. Dose-dependent recovery of pigments (left) and osmolytes/phenolics (right) with GA_3 under salt stress.

Correlation structure of the dataset

Pearson correlation mapping across all treatments (Figure 5) exposed a highly coordinated response system. GA_3 dose correlated strongly and positively with plant height (r = 0.93), leaf length (r = 0.96), phenolics (r = 0.88), sugar (r = 0.85) and protein (r = 0.82), whereas $KMnO_4$ showed only weak positive correlations with these traits because the stress axis was largely offset by GA_3 in the combined dataset. Growth and biochemical markers were themselves tightly inter-correlated (r frequently > 0.90), indicating that GA_3 acts on a linked physiological network rather than on isolated traits. This coordinated structure is precisely the kind of pattern that mean-comparison tables tend to obscure but correlation mapping makes explicit.

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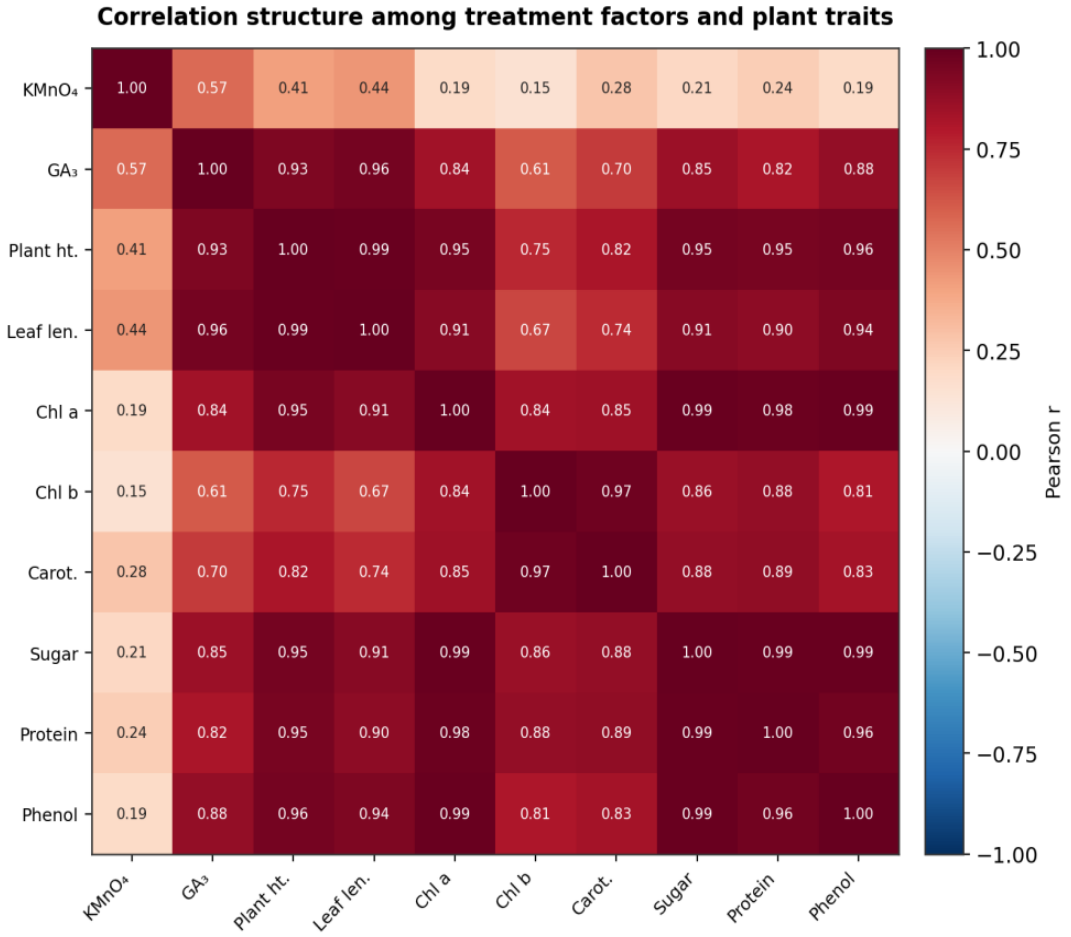

Figure 5. Pearson correlation matrix of treatment factors and measured traits, revealing GA_3-linked coordination.

Machine learning attribution and predictive modelling

Random forest feature attribution ranked GA_3 dose as the leading positive driver of both plant height and protein content with the $KMnO_4 \times GA_3$ interaction term a close second and the standalone $KMnO_4$ stress term contributing least (Figure 6). This quantitatively confirms the physiological narrative: within the treated range, GA_3 supply, both directly and through its interaction with the stress level, governs recovery, whereas residual stress alone is the weakest predictor. A gradient-boosted regression relating treatment inputs to protein content, evaluated under leave-one-out cross-validation, achieved $R^2 = 0.64$ and Pearson $r = 0.80$ (Figure 7), showing that a compact two-factor model already explains a substantial share of the biochemical variance from treatment settings alone. While the dataset is deliberately small and these models are illustrative rather than production-grade, they demonstrate how interpretable ML converts routine agronomic tables into ranked, testable hypotheses about which levers matter most, an approach readily scaled to larger phenotyping datasets.

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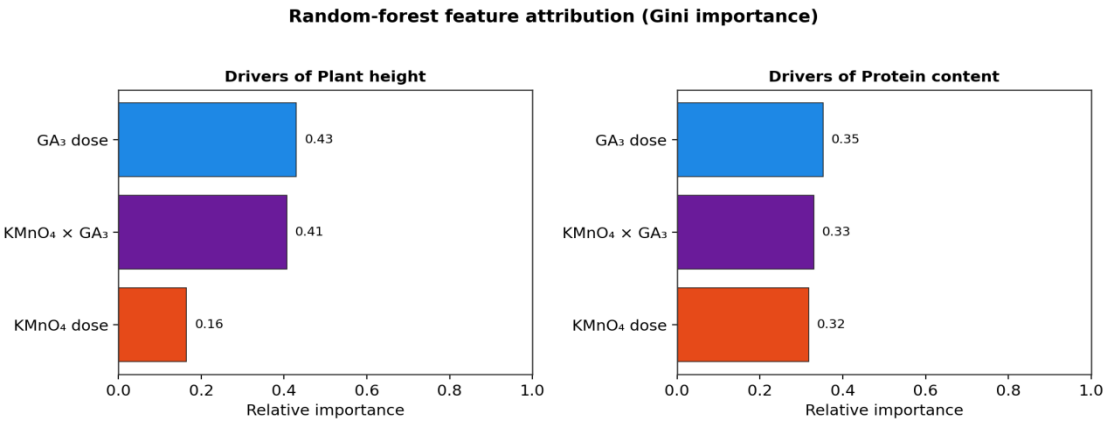

Figure 6. Random-forest (Gini) feature importance identifying GA_3 dose as the leading predictor of growth and protein status.

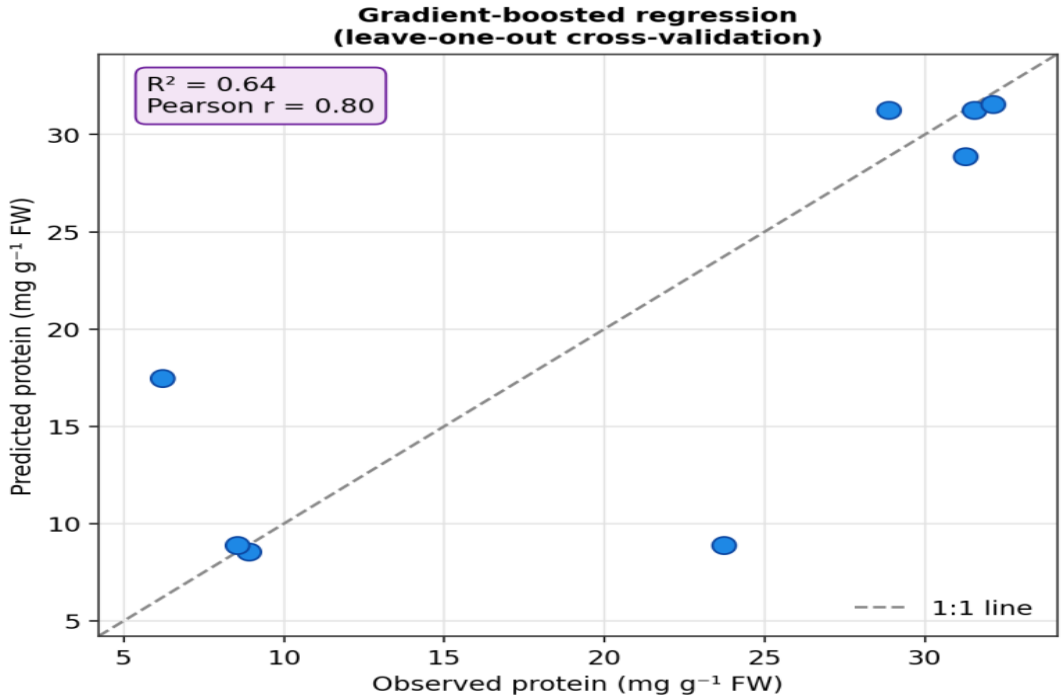

Figure 7. Predicted vs. observed protein content under leave-one-out cross-validation (gradient-boosted regression)

CONCLUSION

Oxidative salt stress caused by $KMnO_4$ significantly affected the morphological and biochemical performance of R. sativus L. by affecting the plant height, leaf growth, fruit growth, photosynthetic pigments, soluble sugars, protein and total phenolics. Much of this damage was reversed by a treatment with exogenous gibberellic acid (GA_3), in a clear dose-dependent manner: the 200 ppm GA_3 + 40 µM $KMnO_4$ combination brought the pigment pools back to the stressed control level and increased the levels of protein and phenolics, in agreement with the stabilizing effect of GA_3 on membranes and the stimulation of protein and osmolyte biosynthesis. The interpretable

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machine-learning layer independently validated these findings to show that the strongest positive predictors (correlation, feature importance) are the GA_3 dose and that it can generate a cross-validated regression that is predictive of biochemical status based on treatment inputs. In combination, the findings suggest that GA_3 is a promising strategy to reduce salt-induced oxidative stress in radish and demonstrate the potential of using a simple, clear computational workflow to augment the inferences of traditional plant-stress studies. Future research should involve expanding the data set to cover additional genotypes and field seasons for the development of more powerful predictive models and precision management of saline soils.

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