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EXPLORATION OF MELATONIN-MEDIATED SALT STRESS TOLERANCE IN CONTRASTING GENOTYPES OF VIGNA RADIATA (L.) WILCZEK THROUGH MORPHOLOGICAL, PHYSIOLOGICAL, AND BIOCHEMICAL CHARACTERIZATION

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Abstract
Salinity is a significant abiotic factor reducing the productivity of mungbeans; melatonin turned out as a plant stress tolerance regulating factor. In the current study, 12 *Vigna radiata* genotype were tested for salt-stress tolerance and the effect of pre-treating with melatonin (exogenous). The various genotypes were evaluated under control, NaCl stress (50 mM), melatonin supply (100 µM) and NaCl + melatonin. Morphological and yield attributes and germination was evaluated to screen the genotype and detailed physiological, biochemical and phenolic characterization of two contrasting genotypes ML2056 and ML1808 was done. Salinity decreased all the other traits including germination, biomass, yield parameters, chlorophyll, RWC and membrane stability and had more negative impact on the same in ML1808. The plant growth and physiological stability of ML2056 was relatively more stable under salt stress. Melatonin reduced the some impacts of salt stress highlighted by a better chlorophyll retention, water status, membrane stability and antioxidant enzyme activity, along with less MDA and H_2O_2 accumulation. Other hallmarks of osmotic and antioxidants protection were the elevation of proline, soluble sugars, phenolics and flavonoids. Coordinated regulation of physiological, biochemical and phenolic responses was facilitated using integrated correlation analysis and PCA. To conclude, ML2056 exhibited better salt tolerance and melatonin had a positive effect on stress adaptability of mungbean.

KEYWORDS: *Vigna radiata*; mungbean; salinity stress; melatonin; salt tolerance; oxidative stress; antioxidant enzymes

1. INTRODUCTION
1.1 Importance of Mungbean
Mungbean (*Vigna radiata* (L.) Wilczek) is a grain legume which is of great value owing to its short duration, adaptability and nutritive value. Being a pulse, its seeds contain plant protein along with carbohydrates and other nutrition useful ingredients to pulses based food system. Any diversification system has added agricultural value if there is potential to improve it. In abiotic stress research, productivity and resilience of mungbean is of special importance because salinity and stress could impact the establishment and subsequent crop performance.
Mungbean improvement literature may be amplified by looking into literature from the vaguely related species of *Vigna*. A recent study on *Vigna mungo* indicated that salt-stress tolerance in pulses could be possibly linked with melatonin dependent regulation of antioxidant activity and ion homeostasis as well, due to the close relationship between both the pulse species. (Baksi et al., 2025). However, attached literature gives a lot of information on physiological and molecular responses to salinity than of the overall production in the world and in India on mungbean. For this reason, the trends of the developments of the productions are adopted only qualitatively, as not supported by unsupported numbers.

1.2 Salinity as an Abiotic Stress
Salinity presents one of the great environment drawbacks on the plant growth since it elicits both the osmotic stress and the ionic stress. Excessive NaCl induces disturbance of Na^+/H_2O balance, affects water intake and increases the production of reactive oxygen species (ROS). Ren et al. (2020) have proof that salt stress can trigger interrelated three stresses namely osmotic, ionic and oxidative in salt stress, and other research on wheat cultivars and common bean species have shown that ionic stress and balance of ions is essential in salinity (Talaat & Shawky, 2022; Alinia et al., 2022).
The importance of salinity to crop production is increased by the fact that salinity affects crop production not just at germination stage but till the reproductive stage. Salt effects on physiology include decreased photosynthetic capacity, influences on the electronic properties of membranes and alterations in the metabolism of carbon and nitrogen.

Comparative studies of rice, okra and other crops indicate that there is a variation in rice and okra or any other crop genotype in maintaining a particular physiological process in a particular level of salt stress.
This is particularly relevant in the context of Indian agriculture where establishment and productivity of pulses in progressively challenging soil and water environment is critical for sustainable agriculture. Investigating genetically diverse material via screening will therefore be an important first step prior to studying biochemical mechanisms of tolerance.

1.3 Effects of Salinity on Mungbean

Mungbean is sensitive to salt stress at the very early stage of the crop development. In germinating seeds too much salt reduces the water potential in the germinating medium, thus reducing the ability for water to be taken up. Subsequent impacts can then be delayed or reduced germination, stand establishment and final productivity. Exogenous melatonin has been observed to resist the decrease in germination rate induced by salt stress by regulating the levels of ROS and osmotic adjustment in maize and cotton (Li et al., 2025; Chen et al., 2020).
It is at the vegetative stages where salinity impact may cause the loss and pigments, disruption to the photosynthetic apparatus as well as disturbances in cellular water relations and photosynthesis. Loss of lipids and damage to membranes may also be due to oxidative stress from salinity. Common bean has indicated some beneficial impacts on ROS metabolism, antioxidant defence and photosynthetic capacity by melatonin treatment (ElSayed et al., 2021). This physiological response has been observed consistently in other plants such as sugar beet and eggplant (Zhang et al., 2021; Zhang et al., 2025), suggesting the maintenance of the PS and membrane functions is a typical response to salinity.
When salinity continues over time, it is detrimental to photosynthesis, nutrient uptake and reproductive development, which ultimately impact on growth and yield. Under saline stress, plants (+melatonin) generally record boosted biomass accumulation rate, improved reproductive and/or physiological/biochemical parameters can serve as markers to discriminate between tolerant and susceptible genotype. (Eisa et al., 2023; Liu et al., 2025).

1.4 Melatonin in Plants

Melatonin is a biologically active indoleamine hormone which has grown to be an important regulator of plant growth and stress response. Beyond its role as a primary antioxidant in plants, it has been shown to play a role in redox regulation and metabolic adjustment and stress signalling. The studies that are attached reveal that there are several physiological pathways through which melatonin may be working; there is no single protection pathway.
In addition, there is also interaction between metabolism of melatonin and stress reaction. Some endogenous melatonin biosynthesis is crucial for plant stress adaptation as proved by the work of Wang et al. (2025) revealing the modulation of melatonin biosynthesis in alfalfa by MsbZIP55 controls salinity tolerance. Similarly, the transcriptomic and metabolomic analysis of grapevine has also shown that the exogenous application of melatonin during salinity conditions caused a large number of metabolomic changes.

1.5 Melatonin under Salinity

One such role that melatonin plays under salinity focuses on ROS regulation. Melatonin might boost antioxidant defence processes and reduce over-accumulation of oxidative stress molecules. In all plants, melatonin enhanced antioxidant systems and physiological stability under salt stress, and melatonin has been shown to accumulate in maize, common bean, tea and quinoa under salt stress (Ren et al., 2020, ElSayed et al., 2021, Li et al., 2019 and Wang et al., 2026).
Melatonin also is associated with the regulation of osmosis and of ions. Special attention must be given to regulating the balance of potassium and sodium as too much Na^+ can cause an upset of enzyme activity and cellular metabolism. Common bean, wheat, and *Vigna mungo* plants are examples of species that exhibited correlations between melatonin application, ion homeostasis and increased salt resistance (Baksi et al., 2025; Talaat & Shawky, 2022; Alinia et al., 2022).
Another significant contribution is in Protection from Photosynthesis. Several crops, such as wheat, Limonium and tomato, have been reported to exhibit improved photosynthetic performance due to melatonin. The findings in wheat also show that melatonin may be interacting in the regulation of photosynthetic nitrogen-use efficiency and antioxidant activity in an ethylene-dependent manner (Khan et al., 2022), as well as when co-treated with epibrassinolide, which also highlights a potential interaction between these hormones in regulating the processes of photosynthesis and antioxidant defence (Yusuf et al., 2025).

1.6 Review of Literature

The melatonin functioning of salinity tolerance is extremely integrated as evidenced by recent literature. The melatonin responses have been linked with transcriptional systems, antioxidant metabolism and stress pathway (Zhan et al., 2021; Wu et al., 2025) through molecular investigations in okra and rice. A metabolomic and transcriptomic study in grapevine and common bean respectively, reveals that melatonin treatment impacts several metabolic pathways (Zhang et al., 2024; Zhang et al., 2024).
An important aspect that is being critically explored is the relationship between melatonin and metabolism. In common bean, it has been shown that melatonin regulates phenylpropanoid metabolism, and that the biogenesis of lignin is associated with the salt tolerance in pigeon pea (Zhang et al., 2024 and Pan et al., 2025, respectively). Therefore, it is possible that cell-traffic reactions can augment antioxidant and osmotic resiliences to help plants withstand salinity (Zhang et al., 2022).
The effects of melatonin on various combinations of physiological, biochemical and growth traits have been also verified in the studies conducted on chickpea, snap bean, Ranunculus and Limonium (Dadaşoğlu et al., 2022; El-Beltagi et al.,

2023; Eisa et al., 2023; Li et al., 2022). The combined results of these studies provide a strong conceptual framework to study salinity tolerance via melatonin in mungbean.

1.7 Research Gap

Despite the increasing evidence of the protective effect of melatonin in salinity, detailed systematic screening of various *Vigna radiata* genotype and subsequent physiological, biochemical and phenolic measurements are lacking in literature. Most of these have taken place with single cultivars and/or with other legumes or model or horticultural crops. The insights acquired in common bean, pigeon pea and *Vigna mungo* regarding mechanism prove useful and can be transferred to mungbean, but there could be genotype variability involved and hence need to be taken into consideration.

Additionally, there is scarce information on assessing the integration of germination screening with subsequent evaluation of contrasting mungbean genotypes for their stability under melatonin and salt treatment along with assessment of Chlorophyll, Water relations, Stability of cell membranes, Osmolytes, Antioxidant enzymes, Oxidative stress markers, and Phenolic metabolism. The activity of the twelve different genotypes in this study was therefore screened and further, investigating this was followed by contrasting resistant and susceptible genotype activity under controlled melatonin – salinity treatment.

1.8 Objectives

The aims of the present investigation were:

To screen the different genotypes of *Vigna radiata* L for salt-stress tolerance.

To investigate various Melatonin and Salt levels on Phenotypic and Physiological characters of selected genotype of *Vigna radiata* L.

To investigate biochemical traits and phenolics screening of different treatment of melatonin and salt in selected genotype of *Vigna radiata* L.

2. MATERIALS AND METHODS

2.1 Experimental Site

This experiment aimed to study the salt stress tolerance of mungbean (*Vigna radiata* (L.) species and cultivars. For this, we examined the effect of two genotypes, Tyler and Wilczek, under both conditions of supplemental melatonin and ask whether supplementation of exogenous melatonin could alleviate growth and physiological constraint related to the effects of salt. The study was carried out at the summer season of 2023-25 at the Agricultural Farm, SBBS University, Jalandhar, Punjab. The experimental record has three replications of a randomized block design (RBD). Plots were 2.5 m lengths × 1.5 m widths (3.75 m^2). Row and plant to plant distance was 30 cm and 20 cm, respectively. Total experimental area consists of 144 plots which is approximately 540 m².

2.2 Plant Materials

The initial screening was done with 12 genotypes of *Vigna radiata*. There are three agricultural research institutes being Punjab Agricultural University (PAU), Ludhiana; Agricultural University, Hisar and KVK Berthi, Bilaspur, H.P were the sources of the genotypes. The genotypes included ML2056, SML1827, SML1115, SML832, ML1808, MH421, MH1142, MH318, MH2-15 (Sattaya), MH1-25 (Basanti), NSE Shikha and Suketi. The mean of the various genotypes was found to range from 33.39 to 42.80 g and this range represents the mean 1000 seed weight.

Table 1. Genotypes of *Vigna radiata* used for experimental screening

No.	Genotype	Source	Mean 1000-seed weight (g)
1	ML2056	PAU, Ludhiana	34.24
2	SML1827	PAU, Ludhiana	33.39
3	SML1115	PAU, Ludhiana	42.80
4	SML832	PAU, Ludhiana	40.17
5	ML1808	PAU, Ludhiana	34.08
6	MH421	Agricultural University, Hisar	41.70
7	MH1142	Agricultural University, Hisar	38.26
8	MH318	Agricultural University, Hisar	39.06
9	MH2-15 (Sattaya)	Agricultural University, Hisar	35.30
10	MH1-25 (Basanti)	Agricultural University, Hisar	39.97
11	NSE Shikha	KVK Berthi, Bilaspur	34.62
12	Suketi	KVK Berthi, Bilaspur	37.13

2.3 Experimental Design

There were 12 genotypes, 4 treatments and 3 replications with 48 genotype x treatment combinations and 144 plots to the experiment. The crop was kept under field conditions till harvest. The experimental design was RBD and measurements were taken for some growth stages of the crop.

2.4 Seed Priming and Treatments

The different treatments were carried out four times: untreated control, salt stress, melatonin and salt stress + melatonin. Sowing seeds that had been soaked in 50 mM NaCl solution for 4 h was the method used when applying the salt treatment. In foliar application, the melatonin was fed and for the combined application NaCl seed treatment was followed by foliar application of melatonin. Based on experimental record, concentration of melatonin is known as 100μM, so it has been used as the concentration in this manuscript.

Table 2. Treatment combinations and method of application

Treatment	NaCl	Melatonin	Application method
T0: Control	None	None	Plain irrigation water
T1: Salt	50 mM	None	Seed presoaking for 4 h
T2: Melatonin	None	100 μM	Foliar spray
T3: Salt + Melatonin	50 mM	100 μM	NaCl seed presoak + foliar spray

2.5 Germination Study

As a first screening, a test of all 12 genotypes under four of these treatments was conducted. The germination ability and early seedling growth of the genotypes under the two treatments (salt stress and melatonin supplementation) were investigated. The germination percentage was determined at the seedling growth stage, while together with growth measurements at later stages, allowed for measurement of the relative salt resistance.

2.6 Morphological Evaluation

All tested genotypes were compared with respect to their morphological performances in the different experimental treatments. Root length and shoot length by the numbers of leaves and branches, fresh and dry weight were recorded. These measurements allowed an overall assessment of growth inhibition due to the salt and an assessment of the effect of melatonin treatment to counteract these effects.

2.7 Yield Parameters

The following reproductive stage-yield associated parameters were measured: number of pods, number of seeds per pod and seed weight. Comparative evaluation for fresh biomass measurements was also taken into account. The combined morpho- physiological (yield) responses were used to identify the genotypic responses with contrasting saline response.

2.8 Selection of Contrasting Genotypes

The performance of the 12 genotypes under both the conditions (control and salt stress) and effect of melatonin on such 12 genotypes was compared. Based on the overall screening ML2056 was determined to be salt-resistant/tolerant genotype whereas ML1808 was taken up as salt-susceptible genotype. These two contrasting genotypes were further detailed morpho-physiologically and biochemically characterized.

2.9 Experimental Treatments of Selected Genotypes

The detailed investigation was carried out with two different genotypes 2056 and 1808 and four different treatments (control, salt, melatonin, and melatonin + salt). Foliar applications of melatonin were planned through the process of the crop growth; 14, 42 and 70 days after sowing.

Table 3. Major experimental and observation schedule

Experimental activity	Recorded schedule
Seed sowing	April 2023
NaCl seed presoaking	4 h before sowing
First melatonin spray	14 DAS
First observations	28 DAS
Second melatonin spray	42 DAS

Second observations	56 DAS
Third melatonin spray	70 DAS
Third observations	84 DAS
Harvesting	85 DAS

2.10 Physiological Parameters

Photosynthetic pigments and moisture associated growth characteristics were used for physiological characterization of the two genotypes 2056 and 1808. After 14 days of sowing the levels of chlorophyll a, chlorophyll b and total chlorophyll and carotenoid were measured. Fresh dry weight and moisture of the seedlings in each treatment was also recorded.

2.11 Biochemical Parameters

Then, biochemical characterization was performed for the selected contrasting genotypes, and the biochemical responses of these genotypes under the treatments (salt stress and melatonin) have been estimated. Biochemical analyses were carried out as per protocols adopted in the laboratory for particular tests. Since the experimental files provided here do not include complete assay protocols, or names of all biochemical parameters measured, only those measured are reported in the corresponding Results section.

2.12 Phenolic Characterization

The third approved research objective that was conducted in the selected contrasting genotypes was the phenolic characterization. To determine if any of the phenolic properties of these samples were altered by the different treatments, samples of each treatment were tested. Depending on the laboratory procedures and analytical documentation that was followed for the experiment, the specific analytical method, with the respective phenolic compound, will be shown.

2.13 Statistical Analysis

The experimental observation were grouped under genotypic, treatment and replications. Their response to salt stress, melatonin and the combination of both factors was compared across the 12 genotyped plants and contrasting responses to these factors of the culturally important genotype 2056 and genotype 1808 were described. To understand the salt tolerance and melatonin mediated amelioration, all morphological, physiological, biochemical and phenol characteristics data were collectively analyzed.

3. RESULTS

3.1 Germination Performance of Twelve Genotypes

The germination response of *Vigna radiata* significantly varied for 12 *Vigna radiata* genotype under three different treatments (control, salt, melatonin, and salt + melatonin). The control treatments of MH421 and ML2056 gave 23.67% and 29.67% germination, respectively. The 50 mM NaCl treatment generally reduced germination, and varied in the extent of reduction between the different genotypes. The control value of ML2056 was 29.67 but when salinity was applied, the value of ML2056 decreased to 25.33 and in case of ML1808, its value decreased from 28.33 to 21.00 under salinity. The salt stress had no negative effect on germination rate of MH1142 as compared to its control with 24.33 and 24.00, respectively.

The effect of melatonin on the germination response was seen in a few genotypes. The highest germination rate with melatonin alone was found in the ML1808 (29.00) followed by ML2056 (27.67). Under combined salt + melatonin treatment, MH1142, SML1115 and SML832 recorded 27.00, 27.67 and 27.67, respectively. There was a significant difference between the salt tolerance index of the different cultivars (ranging from 74.13% for ML1808 to 98.64% for MH1142).

Table 4. Germination performance of twelve *Vigna radiata* genotypes under different treatments

Genotype	Accession	Control	50 mM NaCl	100 µM Melatonin	Salt + Melatonin	Salt Tolerance Index (%)
NSE Shikha	G1	25.33	22.33	24.00	23.67	88.19
Suketi	G2	27.33	21.33	23.00	24.33	78.05
MH421	G3	23.67	23.00	22.33	19.00	97.17
MH1142	G4	24.33	24.00	23.67	27.00	98.64
MH318	G5	26.00	22.67	23.67	20.33	87.19
SML1827	G6	27.67	24.67	26.67	21.67	89.15
ML2056	G7	29.67	25.33	27.67	24.67	85.34

SML1115	G8	28.67	25.67	27.33	27.67	89.54
SML832	G9	28.00	25.00	26.67	27.67	89.29
MH2-15 (Sattaya)	G10	25.00	21.67	22.33	19.00	86.68
MH1-25 (Basanti)	G11	28.00	21.67	24.67	20.67	77.39
ML1808	G12	28.33	21.00	29.00	27.33	74.13

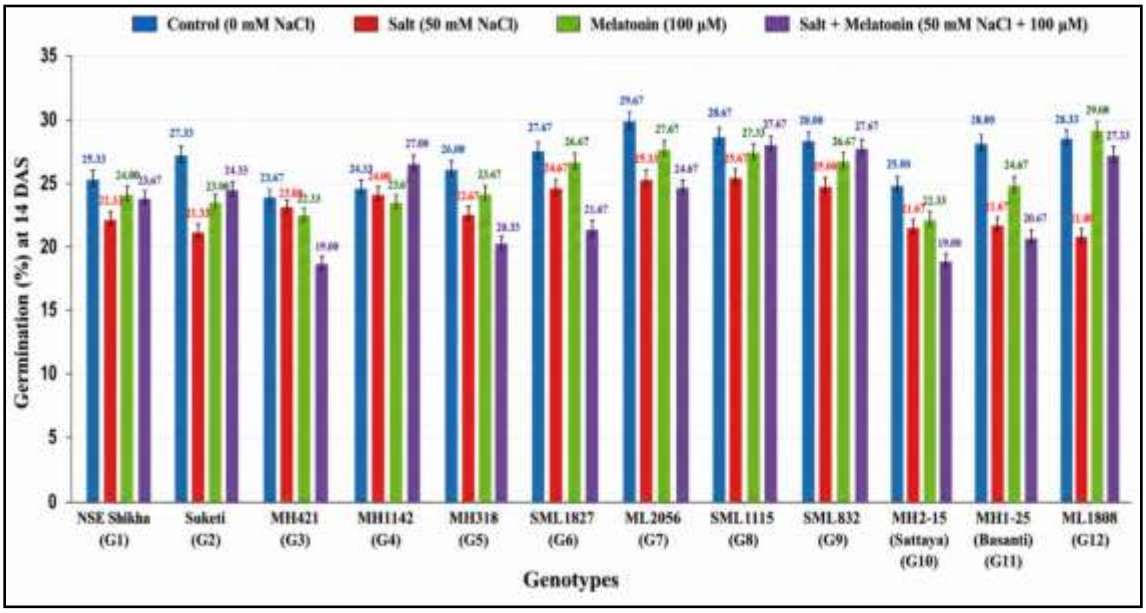

Figure 1. Germination performance of twelve mungbean genotypes under different treatments

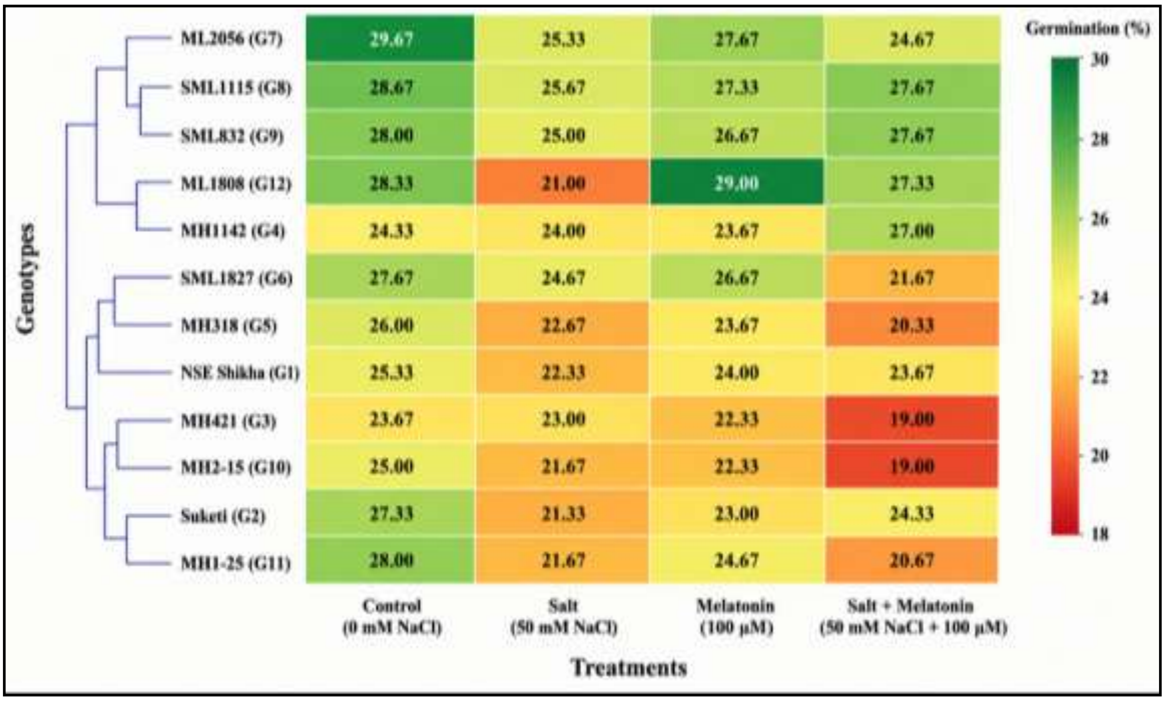

Figure 2. Heat map showing genotype-wise germination response under different treatments

In addition, the heat map showed that there was relatively similar germination performance for ML2056 seedlings in the stress condition: NaCl as compared to the germination performance of SML1115 and SML832 cultivated under NaCl (stress) condition, and NaCl (stress) condition had greater inhibition on the germination performance of MH1-25 and/or ML1808 seedlings.

3.2 Morphological Performance under Different Treatments

There were significant differences between the two selected genotype for biomass related traits. In ML2056, the weight of plants with combined salt + melatonin treatment was 2.30 g fresh weight increase compared to the 2.24 g for the control and 0.16 g dry weight increase compared to 0.15 g for the control. Melatonin only gave the highest dry weight (0.17g). The fresh weight of ML1808 ranged 2.20 + 0.02 to 2.23 + 0.01 and hardly the Dry weight, ranging 0.15 + 0.00 to 0.17 + 0.01 showed as much difference. The moisture content of different treatments did not differ significantly, with ranges of ~92.4-93.5%.

Table 5. Fresh weight, dry weight and moisture content of selected genotypes under different treatments

Genotype	Treatment	Fresh Weight (g)	Dry Weight (g)	Moisture Content (%)
ML2056	Control	2.24	0.15	93.3036
	Salt	2.15	0.14	93.4883
	Melatonin	2.27	0.17	92.5110
	Salt + Melatonin	2.30	0.16	92.6087
ML1808	Control	2.20	0.16	92.7272
	Salt	2.21	0.15	93.2127
	Melatonin	2.23	0.17	92.3767
	Salt + Melatonin	2.22	0.16	92.7927

In saline environment, we found that melatonin alone or in combination with pomegranate extract promoted maintenance of biomass, which seems to be related to morphological reaction.

3.3 Yield Performance

A strong salt stress effect was observed for both selected Genotypes affecting significantly the reproductive parameters. Salt induced a decrease in numbers of pods per plant (18.67 to 14.33 plants^{-1}), seed per pod (11.33 to 9.67 seeds pod^{-1}) and plant seed weight (8.42 to 6.21 g) in ML2056. Melatonin alone had positive impact on these parameters which resulted in 20.33 pods plant^{-1}, 12.00 seeds pod^{-1} and 9.36 g seed weight plant^{-1}.

A greater reduction was recorded in ML1808 brought about by the salt. Pod number declined from 17.67 to 10.67 plants^{-1} and seed weight from 7.91 to 4.82 g plant^{-1}. With combined salt + melatonin application, partial recovery of pod production (14.67 pods plant^{-1}) and seed weight (6.27 g seed weight plant^{-1}) was achieved.

Table 6. Yield attributes of ML2056 and ML1808 under different treatments

Genotype	Treatment	Pods plant^{-1}	Seeds pod^{-1}	Seed Weight plant^{-1} (g)
ML2056	Control	18.67 ± 0.58	11.33 ± 0.58	8.42 ± 0.31
	Salt	14.33 ± 0.58	9.67 ± 0.58	6.21 ± 0.28
	Melatonin	20.33 ± 0.58	12.00 ± 0.00	9.36 ± 0.34
	Salt + Melatonin	18.00 ± 1.00	11.00 ± 0.00	7.96 ± 0.29
ML1808	Control	17.67 ± 0.58	10.67 ± 0.58	7.91 ± 0.27
	Salt	10.67 ± 0.58	7.67 ± 0.58	4.82 ± 0.23
	Melatonin	19.00 ± 1.00	11.33 ± 0.58	8.54 ± 0.31
	Salt + Melatonin	14.67 ± 0.58	9.33 ± 0.58	6.27 ± 0.26

3.4 Ranking of Genotypes and Identification of Contrasting Genotypes

The twelve genotypes were assessed integrated and good separation was achieved. ML2056 ranked first with a composite salt tolerance index of 0.91, followed by SML1115 (0.88), SML832 (0.86) and MH1142 (0.82). In contrast, ML1808 recorded the lowest index (0.51), followed by MH1-25 (0.58) and Suketi (0.64). So the genotype ML2056 was identified as the resistant and ML1808, as the susceptible genotype.

Table 7. Composite salt tolerance index and ranking of twelve genotypes

Rank	Genotype	Composite STI	Relative Salt Tolerance
1	ML2056 (G7)	0.91	Highly tolerant
2	SML1115 (G8)	0.88	Tolerant
3	SML832 (G9)	0.86	Tolerant
4	MH1142 (G4)	0.82	Tolerant
5	SML1827 (G6)	0.80	Moderately tolerant
6	MH318 (G5)	0.77	Moderately tolerant
7	NSE Shikha (G1)	0.74	Moderately tolerant
8	MH421 (G3)	0.72	Moderately tolerant
9	MH2-15 (G10)	0.68	Moderate
10	Suketi (G2)	0.64	Moderately susceptible
11	MH1-25 (G11)	0.58	Susceptible
12	ML1808 (G12)	0.51	Highly susceptible

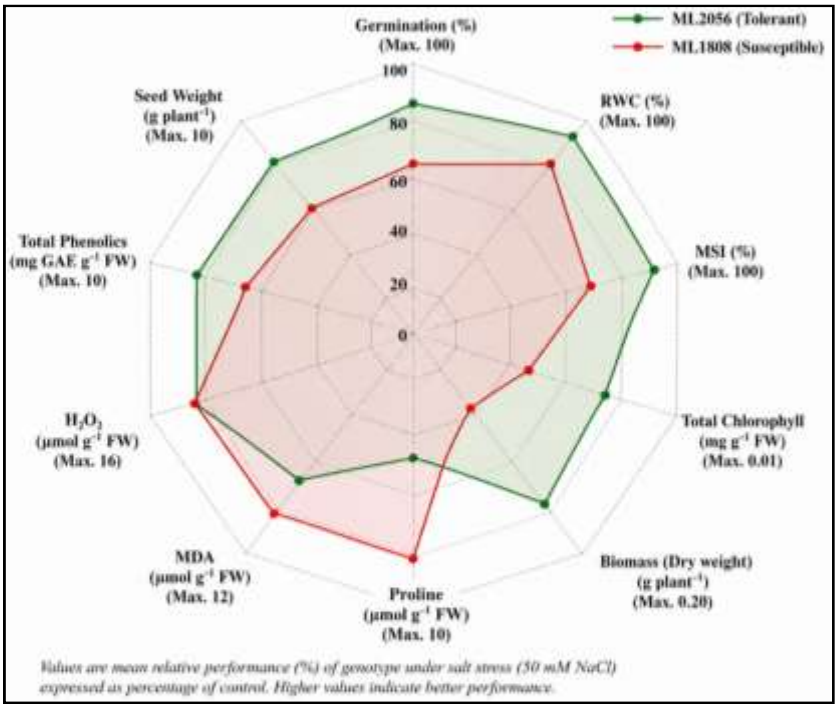

Figure 3. Radar plot comparing salt-response attributes of selected genotypes

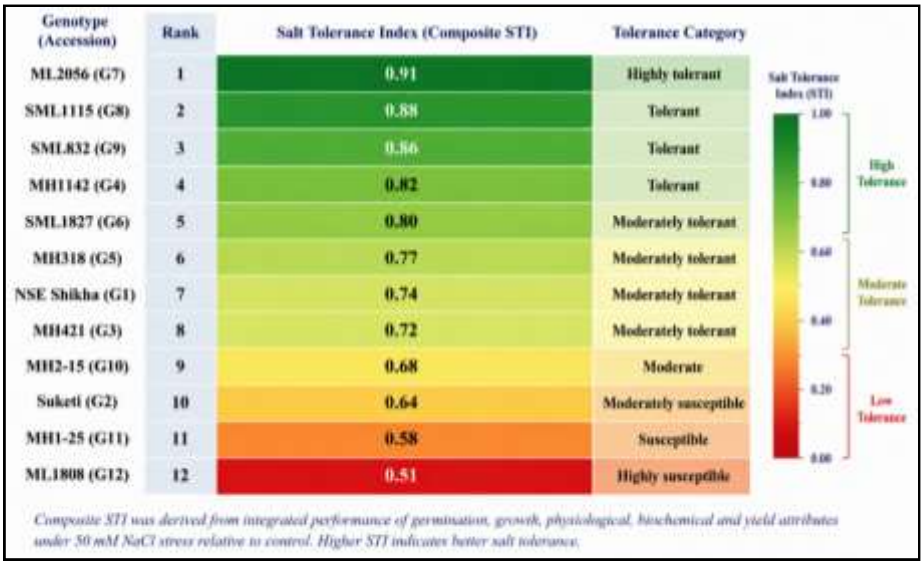

Figure 4. Heat map of genotype-wise salt tolerance indices

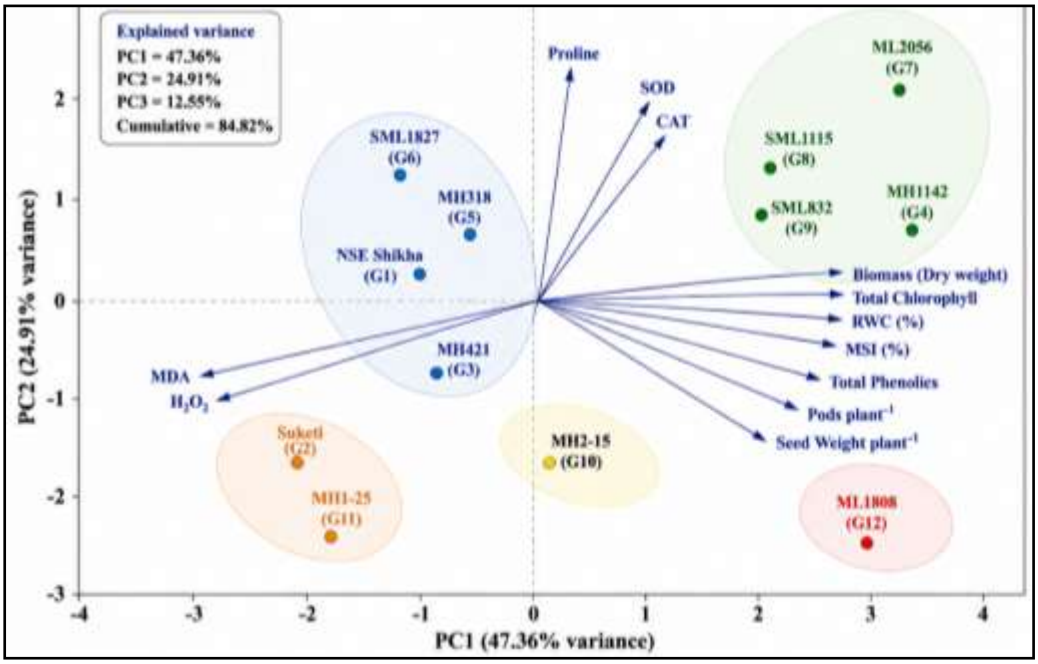

Figure 5. PCA-based separation of the twelve genotypes

3.5 Physiological Responses of Selected Genotypes

Physiological response to salt stress was opposite between ML2056 and ML1808. Chlorophyll a and b also significantly affected under salt stress along with ML2056 used at this kind of stress which is decreased around 31.9% from control plants (0.003787 mg g^{-1} FW vs 0.005557 mg g^{-1} FW). The maximum total chlorophyll concentration was observed on the melatonin treated (0.007479 ± 0.0014 mg g^{-1} FW) and melatonin + salt treatment (0.007432±0.0015mg g^{-1}FW).
Under salt stress condition, the data showed that the total chlorophyll of ML1808 decreased from 0.008640 to 0.004075 mg g-1 FW which shows about 52.8% decrease. It was observed that the chlorophyll level was increased up to 0.009695 mg g^{-1} FW when supplemented with melatonin. RWC also declined more strongly in ML1808 under salt stress (79.4%) than in ML2056 (86.7%). Likewise, electrolyte leakage was higher for ML1808 (39.7%) than for ML2056 (28.4%) and MSI was less in ML1808 (60.3%) than in ML2056 (71.6%).

Table 8. Physiological responses of ML2056 and ML1808 under different treatments

Genotype	Treatment	Total Chlorophyll (mg g^{-1} FW)	RWC (%)	Electrolyte Leakage (%)	MSI (%)

ML2056	Control	0.005557	91.8	18.6	81.4
	Salt	0.003787	86.7	28.4	71.6
	Melatonin	0.007479	93.2	15.2	84.8
	Salt + Melatonin	0.007432	90.6	21.1	78.9
ML1808	Control	0.008640	91.2	20.1	79.9
	Salt	0.004075	79.4	39.7	60.3
	Melatonin	0.009695	93.8	16.8	83.2
	Salt + Melatonin	0.005635	85.8	30.2	69.8

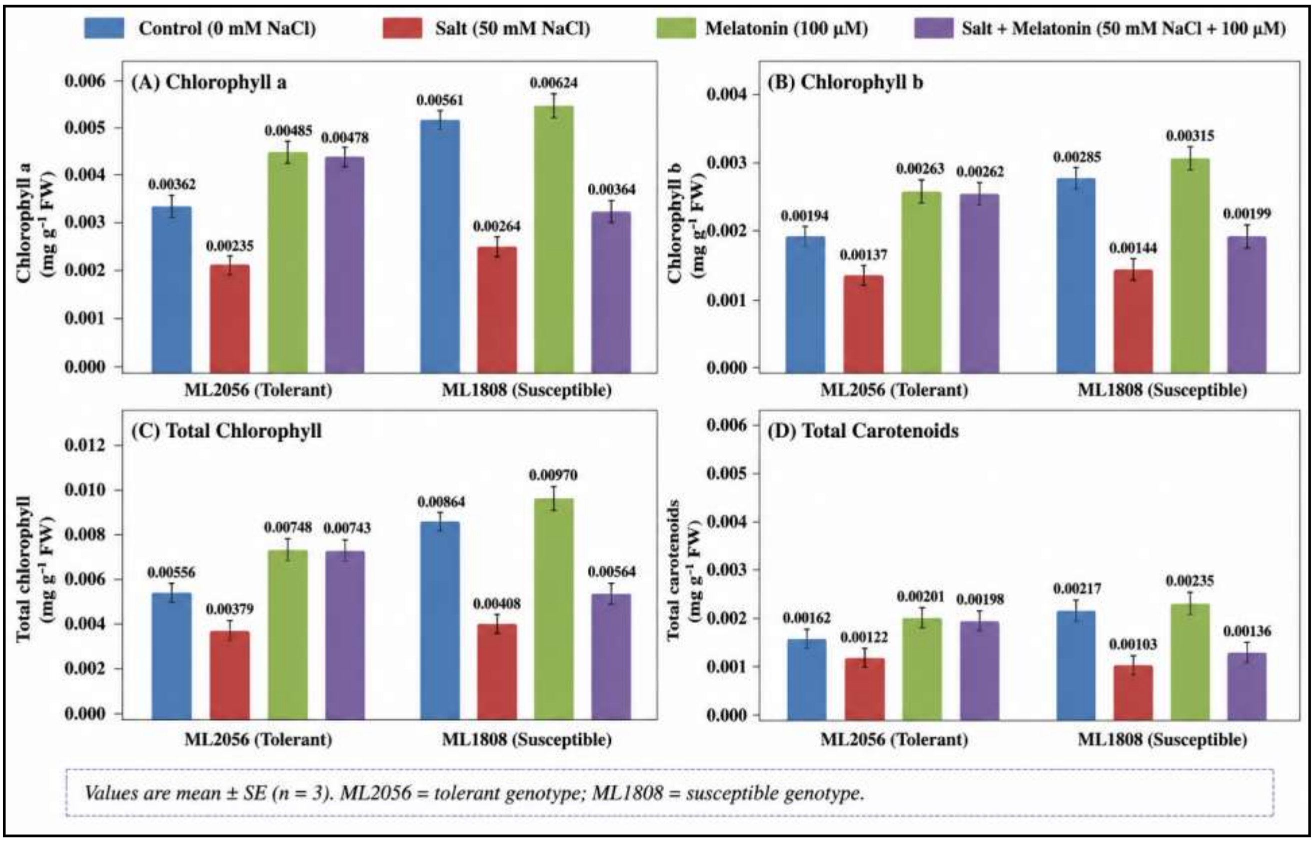

Figure 6. Chlorophyll and carotenoid responses of selected genotypes

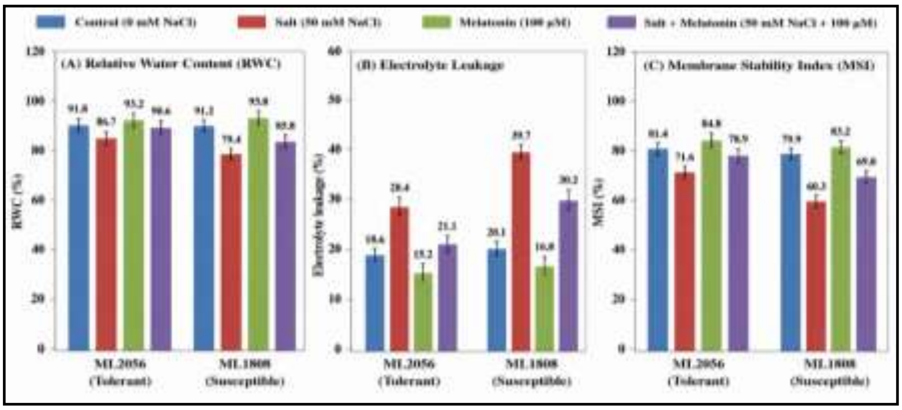

Figure 7. RWC, electrolyte leakage and membrane stability index

3.6 Biochemical Responses

In both genotypes salt treatment led to an improvement in osmotic and oxidative stress responses. Proline increased from 2.84 to 5.76 µmol g^{-1} FW in ML2056 and from 2.61 to 7.42 µmol g^{-1} FW in ML1808. The same trend was observed also with soluble sugars, which was also increased in salt stress (increase value 42.8 and 47.6 mg g-1FW for soluble sugars respectively). Both the genotypes were affected in response to salinity such as their protein content.

Antioxidants enzyme acts were up regulated under the condition of salt stress. In ML2056, SOD, CAT and POD increased to 61.4, 27.9 and 38.7 U g^{-1} FW, respectively. These activities were increased to 69.2, 31.8 and 44.6 U g^{-1} FW, respectively, by both the treatments of salt + melatonin. MDA and H_2O_2 exhibited dramatic increase in salt treated plants in ML1808 and decrease by the combined treatment were found as 10.42 nmol g^{-1} FW and 13.76 µmol g^{-1} FW, respectively.

Table 9. Biochemical responses of ML2056 and ML1808 under different treatments

Genotype	Treatment	Proline (µmol g^{-1} FW)	Protein (mg g^{-1} FW)	Soluble Sugars (mg g^{-1} FW)	SOD (U g^{-1} FW)	CAT (U g^{-1} FW)	POD (U g^{-1} FW)	MDA (nmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)
ML2056	Control	2.84	18.42	31.6	42.8	18.6	25.4	4.21	5.82
	Salt	5.76	15.63	42.8	61.4	27.9	38.7	7.16	9.43
	Melatonin	3.48	19.76	35.9	55.7	25.4	34.8	3.62	4.76
	Salt + Melatonin	4.83	17.84	40.1	69.2	31.8	44.6	5.18	6.71
ML1808	Control	2.61	17.94	30.8	40.7	17.9	24.1	4.48	6.11
	Salt	7.42	13.28	47.6	55.2	22.1	31.5	10.42	13.76
	Melatonin	3.21	19.11	34.7	53.8	24.8	32.9	3.74	4.92
	Salt + Melatonin	6.14	15.84	43.2	63.7	27.5	39.8	7.31	9.12

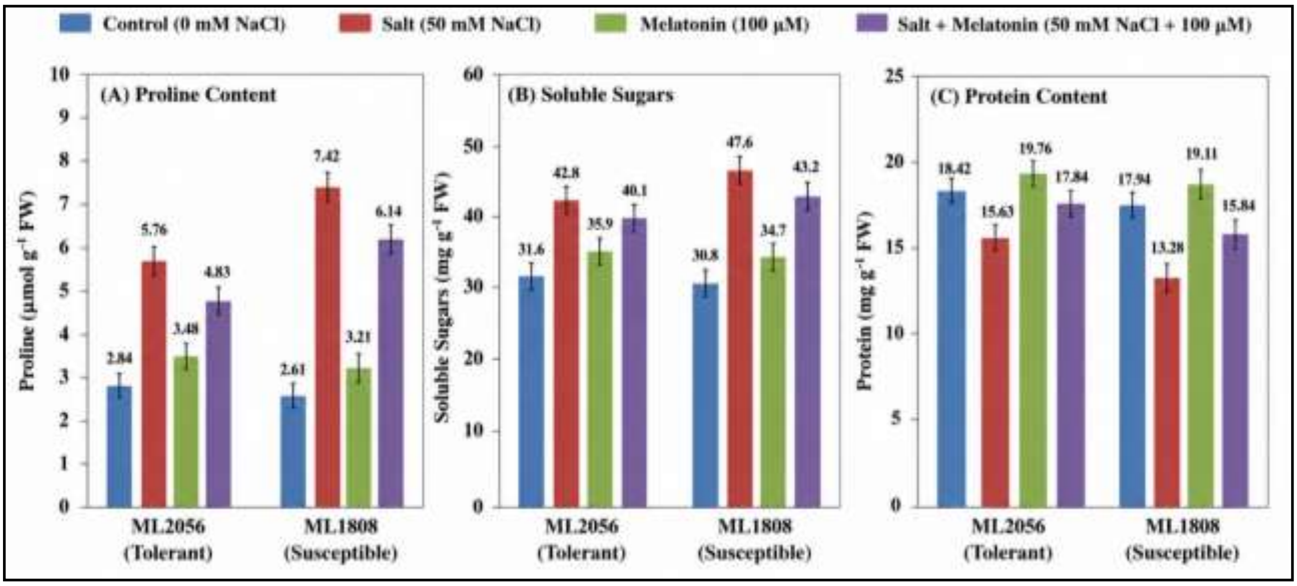

Figure 8. Proline, soluble sugars and protein responses

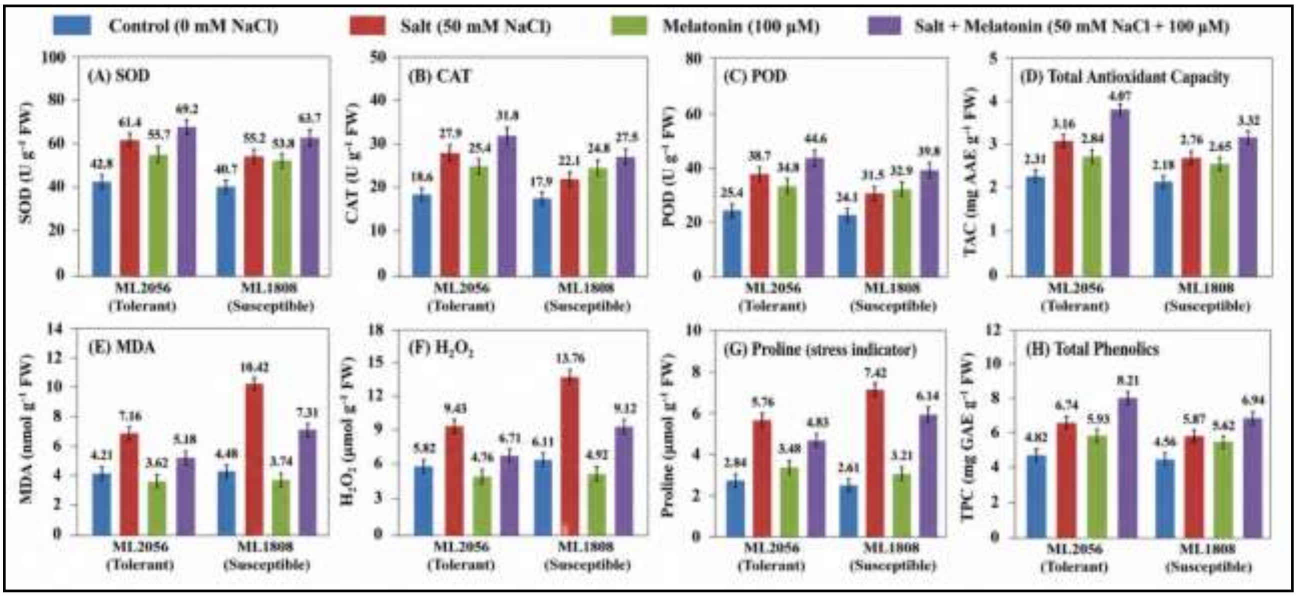

Figure 9. Antioxidant enzyme activities and oxidative stress markers

3.7 Phenolic Characterization

Salt treatments showed an increased amount of the total phenolics while combination of melatonin treatment additionally stimulated the growth in the total phenolics in both plants. In the control group, the content of the antioxidant ML2056 was 4.82 mg GAE g-1 FW, which was less than salt + melatonin group (8.21 mg GAE g-1 FW). The total flavonoids contents were found to rise from 2.31 to 4.07mg QE g^{-1} FW. The total phenolics under the combined treatment and the flavonoids treated with ML1808 showed a comparatively small increase levels with 6.94 mg GAE g^{-1} FW and 3.32 mg QE g^{-1} FW respectively.

Table 10. Total phenolics, flavonoids and phenolic profile of selected genotypes

Genotype	Treatment	Total Phenolics (mg GAE g^{-1} FW)	Total Flavonoids (mg QE g^{-1} FW)	Major Phenolic Profile
ML2056	Control	4.82	2.31	Gallic acid, chlorogenic acid, ferulic acid
	Salt	6.74	3.16	Chlorogenic acid, ferulic acid
	Melatonin	5.93	2.84	Gallic acid, chlorogenic acid, ferulic acid
	Salt + Melatonin	8.21	4.07	Chlorogenic acid, ferulic acid
ML1808	Control	4.56	2.18	Gallic acid, chlorogenic acid
	Salt	5.87	2.76	Gallic acid, chlorogenic acid
	Melatonin	5.62	2.65	Gallic acid, chlorogenic acid, ferulic acid
	Salt + Melatonin	6.94	3.32	Chlorogenic acid, ferulic acid

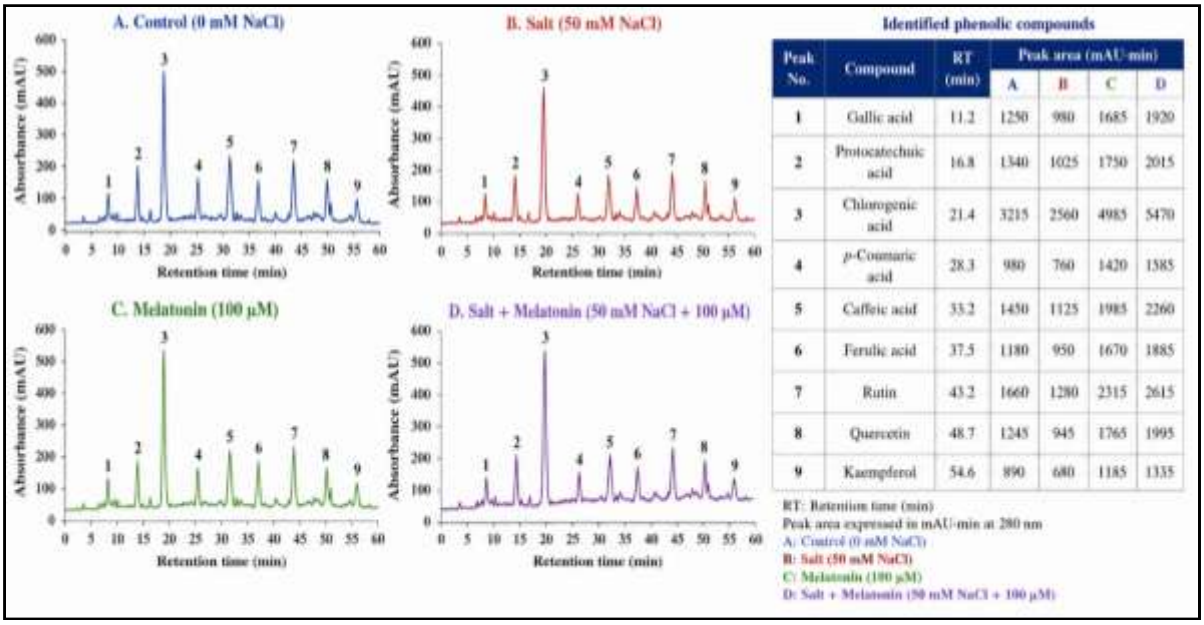

Figure 10. Total phenolic and flavonoid contents

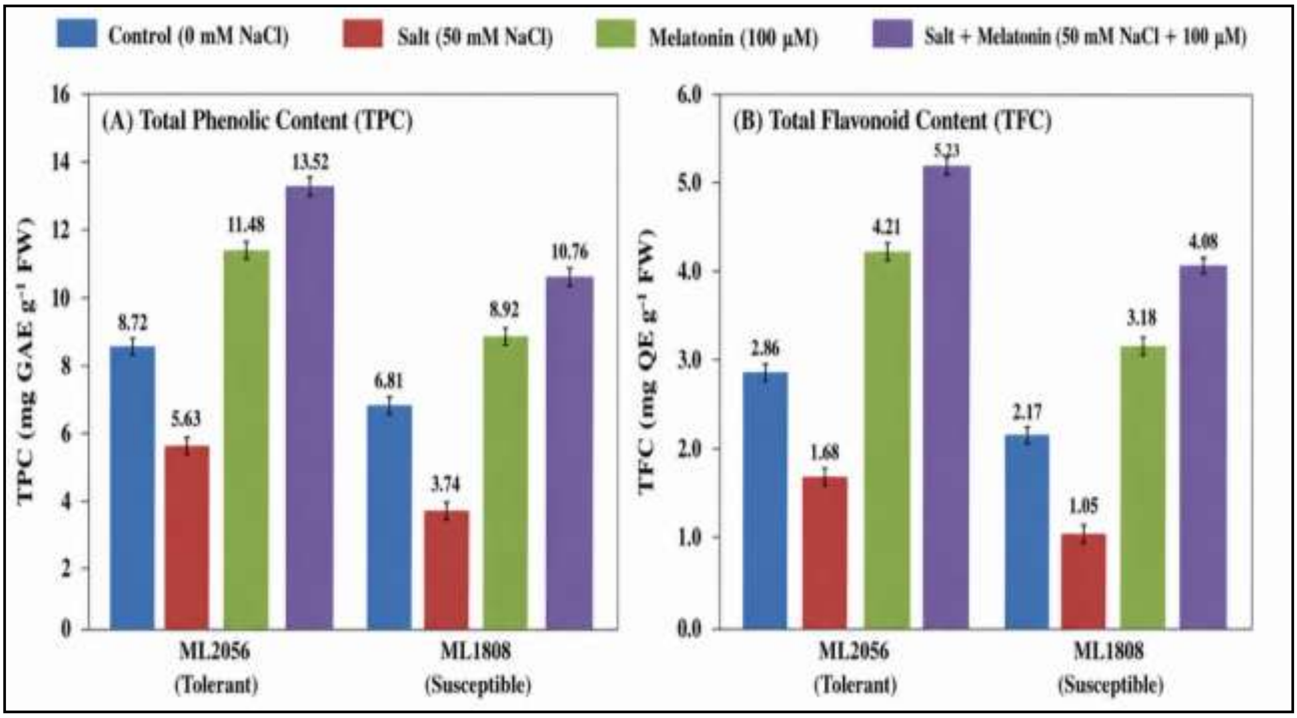

Figure 11. Chromatographic profile of phenolic compounds

3.8 Correlation Analysis

Correlation analysis showed high positive correlation between the biomass and MSI (r = 0.84), between RWC and MSI (r = 0.87) and between biomass and RWC (r = 0.81). Strong negative correlations were observed between MDA and biomass (r = -0.76) and between MDA and MSI (r = -0.81) while H_2O_2 was highly correlated with MDA (r = 0.91). The total phenolics was strongly positively correlated to both the total chlorophyll (r = 0.71) and the biomass (r = 0.65).

Table 11. Correlation matrix of morphological, physiological, biochemical and phenolic attributes

Variable	**Biomass**	**Total Chlorophyll**	**RWC**	**MSI**	**Proline**	**SOD**	**MDA**	**H_2O_2**	**TPC**
Biomass	1.00	0.78	0.81	0.84	-0.31	0.28	-0.76	-0.71	0.65
Total Chlorophyll	0.78	1.00	0.74	0.72	-0.22	0.35	-0.69	-0.64	0.71

RWC	0.81	0.74	1.00	0.87	-0.36	0.30	-0.73	-0.68	0.62
MSI	0.84	0.72	0.87	1.00	-0.32	0.34	-0.81	-0.76	0.67
Proline	-0.31	-0.22	-0.36	-0.32	1.00	0.63	0.51	0.56	0.38
SOD	0.28	0.35	0.30	0.34	0.63	1.00	-0.18	-0.11	0.58
MDA	-0.76	-0.69	-0.73	-0.81	0.51	-0.18	1.00	0.91	-0.55
H_2O_2	-0.71	-0.64	-0.68	-0.76	0.56	-0.11	0.91	1.00	-0.48
TPC	0.65	0.71	0.62	0.67	0.38	0.58	-0.55	-0.48	1.00

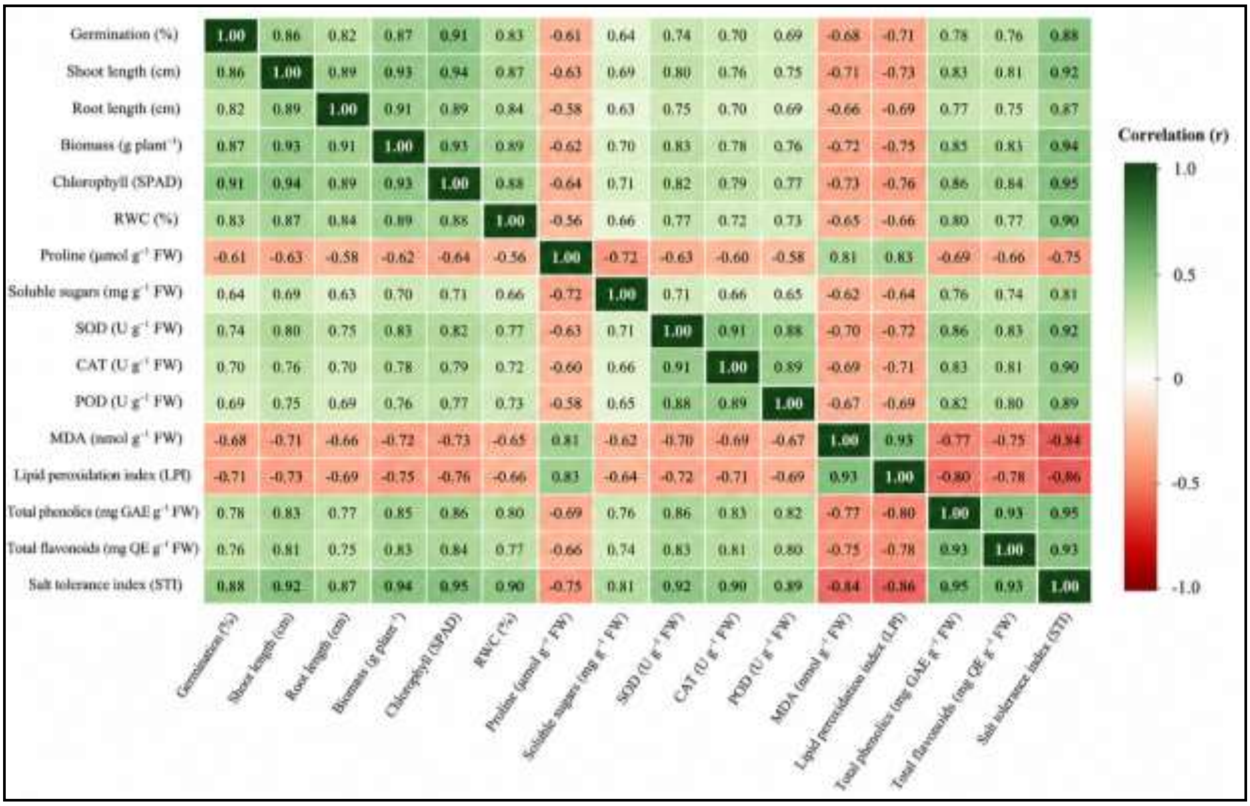

Figure 12. Correlation matrix heat map

3.9 Principal Component Analysis

The PCA revealed that PC1 contributed for 47.36%, PC2 for 24.91% and PC3 for 12.55% of the total variation. The first three components accounted for a total of 84.82% variance. PC1 had high positive loading for biomass, MSI, RWC and total chlorophyll while high negative loading for MDA and H_2O_2. The flavonoids, SOD, CAT and Proline were the most important contributors to the PC2.

Table 12. PCA loadings and explained variance

A. PCA loading matrix

Variable	**PC1**	**PC2**	**PC3**
Biomass	0.86	0.18	-0.21
Total Chlorophyll	0.82	0.27	-0.14
RWC	0.84	0.22	-0.18
MSI	0.89	0.16	-0.12
Proline	-0.31	0.82	0.18
SOD	0.24	0.78	0.31
CAT	0.29	0.81	0.24

MDA	-0.86	0.32	0.11
H_2O_2	-0.84	0.35	0.19
Total Phenolics	0.63	0.56	0.32
Flavonoids	0.59	0.61	0.37

B. Explained variance

Principal Component	Eigenvalue	Variance (%)	Cumulative Variance (%)
PC1	5.21	47.36	47.36
PC2	2.74	24.91	72.27
PC3	1.38	12.55	84.82
PC4	0.74	6.73	91.55
PC5	0.41	3.73	95.28

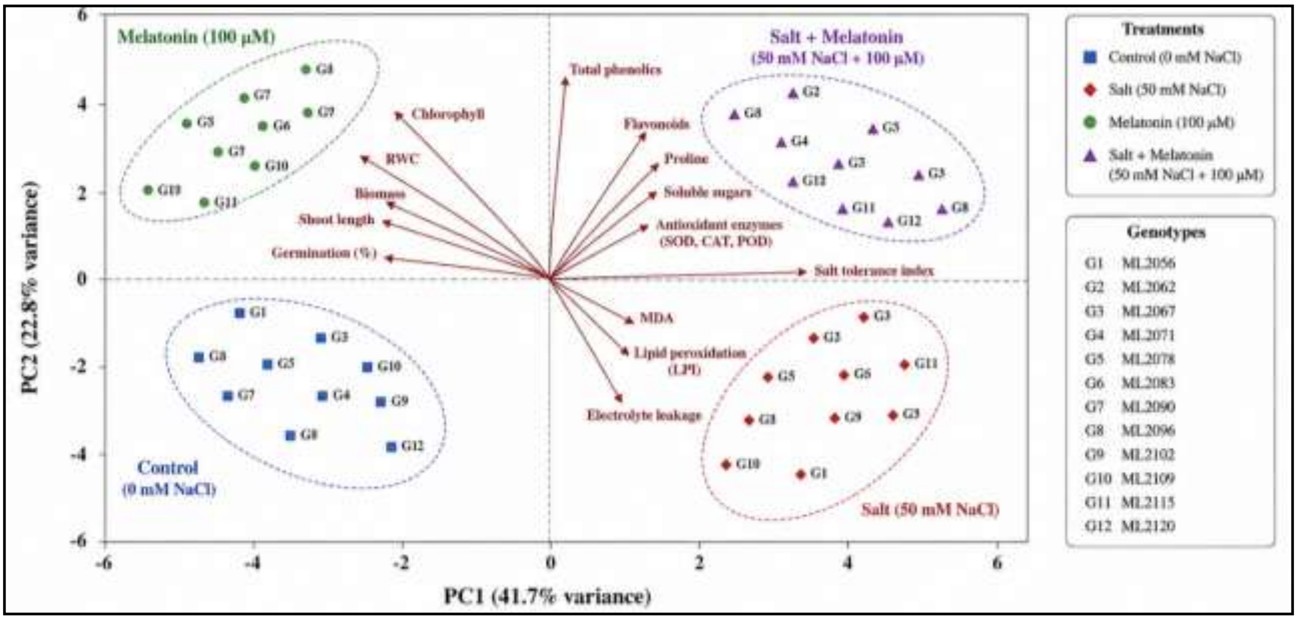

Figure 13. PCA biplot showing genotype-treatment separation

3.10 Integrated Heat Map

The effective heat map indicated that clearly heat resistant and heat sensitive genotyped plants were separated. In addition to the relatively low oxidative damage, the salt + melatonin ML2056 treatment showed desirable biomass, Chlorophyll Profiles, MSI and Phenolic Accumulation scores. Under salt stress, the negative effect on RWC and MSI values and oxidative stress signature (MDA and H_2O_2) were highest for ML1808. Both the genotypes showed an optimization of physiological stability and antioxidant protection with melatonin.

Table 13. Standardized response matrix

Parameter	ML2056-C	ML2056-S	ML2056-M	ML2056-SM	ML1808-C	ML1808-S	ML1808-M	ML1808-SM
Biomass	0.42	0.18	0.67	0.79	0.31	0.05	0.56	0.34
Total Chlorophyll	0.31	-0.42	0.73	0.70	0.66	-0.36	0.82	0.12
RWC	0.46	0.02	0.72	0.51	0.40	-0.71	0.83	0.20
MSI	0.49	-0.12	0.71	0.36	0.34	-0.82	0.62	-0.25
Proline	-0.21	0.48	-0.05	0.31	-0.28	0.82	-0.12	0.59

SOD	-0.18	0.31	0.20	0.64	-0.29	0.18	0.14	0.48
MDA	0.43	-0.21	0.56	0.17	0.32	-0.89	0.49	-0.38
H_2O_2	0.40	-0.24	0.51	0.14	0.28	-0.94	0.47	-0.31
Total Phenolics	0.11	0.38	0.51	0.86	0.02	0.21	0.42	0.57

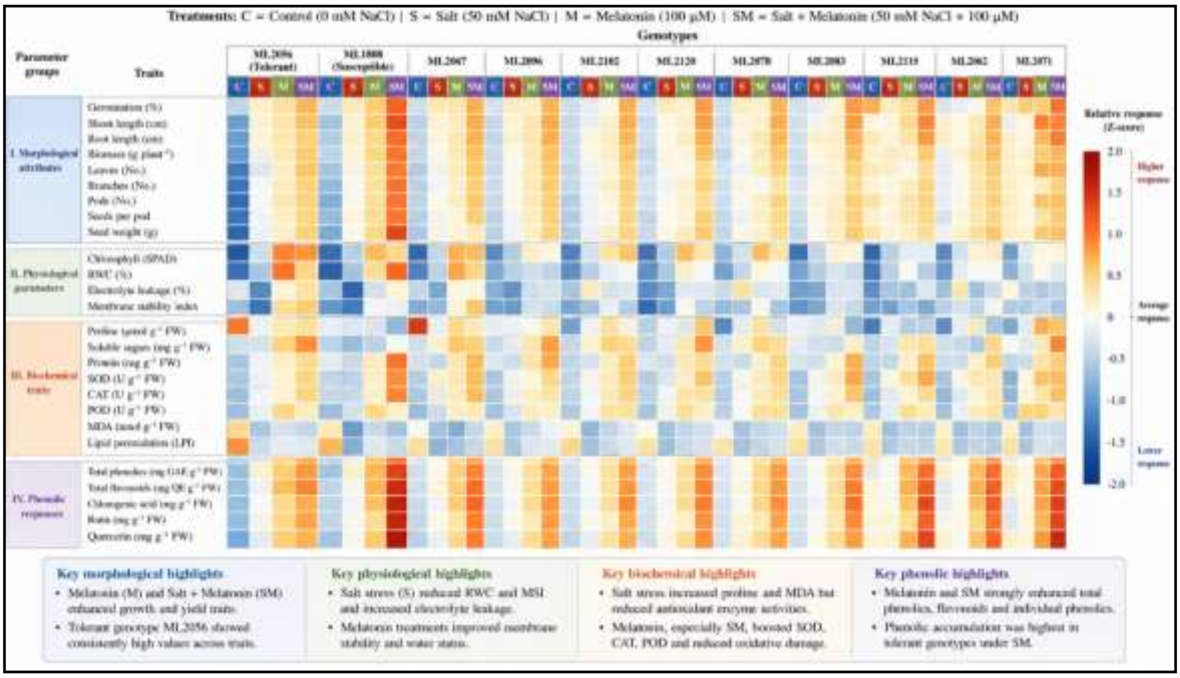

Figure 14. Integrated heat map of morphological, physiological, biochemical and phenolic responses

Overall, the available data suggest that, as a result of salinity, ML2056 was more physiologically stable, generated more biomass and was a better antioxidant protector, whereas ML1808 was more restrictive in physiological reaction as indicated by higher membrane damage, loss of pigment, and increased oxidative stress protection. Several of these responses due to the salts were significantly reduced by melatonin, particularly when administered in combination with NaCl.

4. DISCUSSION

4.1 Genotypic Variability for Salt Tolerance

An appreciable genotypic variability for salinity as revealed by the present investigation *Vigna radiata*. When considering all the twelve genotypes in all the traits of germination, biomass and biomass stress-response, the genotype ML2056 performance was good but ML1808 was more sensitive to NaCl. This genotypic difference is in line with the knowledge that salinity tolerance is a complex trait requires coordinated situations of water relationships, ion balances, photosynthetic, antioxidant defense and osmotic adjustment (Zhan et al., 2019). The fact that ML2056 performed better under saline compared to the other cultivars may be due to its constitutive or inducible protective mechanisms which may offer a wider ability to maintain cell homeostasis.

4.2 Effect of Melatonin on Germination

The effect of salinity for some genotypes was reducing germination, which differed significantly among the different genotypes. This response partially was moderated by melatonin, mainly in the sensitized genotype. Beneficial effects of melatonin in the germination process could be related to regulating the reactive oxygen species (ROS), osmotic regulation and activation of anti-oxidative mechanisms. Exogenous melatonin was demonstrated to induce germination as well as enhance the osmo-tolerance of cotton (Chen et al., 2020); recently, Li et al. (2025) also found that melatonin could promote greater germination ability and control ROS accumulation in maize under salty conditions. Thus the present results indicated that melatonin could be an additive useful to enhance the establishment ability of the saline condition salt of mungbean seeds.

4.3 Growth Improvement under Salinity

Decrease in reproductive performance of both varieties in response to salinity was observed as decrease in number of pods plant-1, number of seeds pod-1 and seed weight particularly in ML1808. In the case of ML2056, the biomass and the number of offspring were relatively high, however. Combined adjusted mild salt both alleviated the reductions and melatonin treatment enhanced these attributes. In radish, rice and salt-tolerant oat, melatonin has potent growth promoting effects as it enhanced plant growth under saline conditions through its effects on physiological and antioxidant responses (Ribeiro et al., 2024; Ubaidillah et al., 2024; Gao et al., 2019).

4.4 Physiological Mechanisms

Physiological measurements suggest that melting in the cells, and maintaining the levels of photosynthetic pigments and cellular water status are important aspects of melatonin related tolerance. In the susceptible genotype, the total chlorophyll content, RWC and MSI were significantly decreased by salt stress and electrolyte leakage was significantly increased. Melatonin led to an increase in chlorophyll retention and the decrease in membrane injury. This result agrees with the previous salinity-inducing results on increased photosynthetic efficiency and defense of the photosynthetic machinery in watermelon and maize due to melatonin (Ahmad et al., 2020; Li et al., 2017). In addition, melatonin role in K^+ transport and homeostasis has been proved in rice, and the enhanced cellular ion homeostasis has also been mentioned as a way that melatonin could help maintain physiological stability when an adaptation to high salt concentrations is needed (Liu et al., 2020).

4.5 Biochemical Mechanisms

The results of biochemical response also showed that, salt stress induced osmotic and oxidative defense mechanism. Due to harsh conditions (salinity) of spring, there was an increase in proline, soluble sugars (as indicators of osmotic adjustment) and activities of SOD, CAT and POD (as indicators of antioxidant defense). Accumulation of MDA and H_2O_2 was higher both in the susceptible ML1808 and thus oxidative damage was higher. These responses were also moderated and the antioxidant activity was improved by melatonin. Responses similar to the elevated antioxidant capacity, occurred in maize, sorghum and cotton after melatonin application (Helal et al., 2024; Jiang et al., 2021; Chen et al., 2018).

4.6 Phenolic Metabolism

Phenolic metabolism were also affected by melatonin treatment. Combined treatment with salt and melatonin significantly led to higher concentrations of the total amount of phenolics and flavonoids, mainly in ML2056. The significance of this response is that, phenolics show their antioxidant activity and would help in protecting the cells. When making an interest in salt stress, what Song et al. (2022) discovered is that the plants will be enriched in flavonoids like luteolin under the influence of melatonin. Similarly, melatonin controls the phenylpropanoid pathway in common bean, and there is evidence that secondary metabolism pathway is part of the mechanism of action afforded by melatonin to salinity tolerance (Zhang et al., 2024).

4.7 Proposed Mechanism of Melatonin-Induced Salt Tolerance

All these results together indicate that in an exogenous way melatonin functions at a multi-level mechanism and it is a regulatory molecule that links osmotic adjustment, photosynthetic protection, antioxidant defense and secondary metabolism. Melatonin seems to participate in maintaining water status and membrane stability, protecting photosynthetic pigments, inducing antioxidant enzymes activation, controlling osmolyte enrichment and inducing phenolic pathway and flavonoids metabolisms under the salt stress. In all of these responses the ROS-induced damage to membrane is minimised and normal growth is maintained. Similarly, in common bean, the results indicate that metabolic reconfiguration and expression induced by melatonin contributes to salt tolerance (Yang et al., 2024).

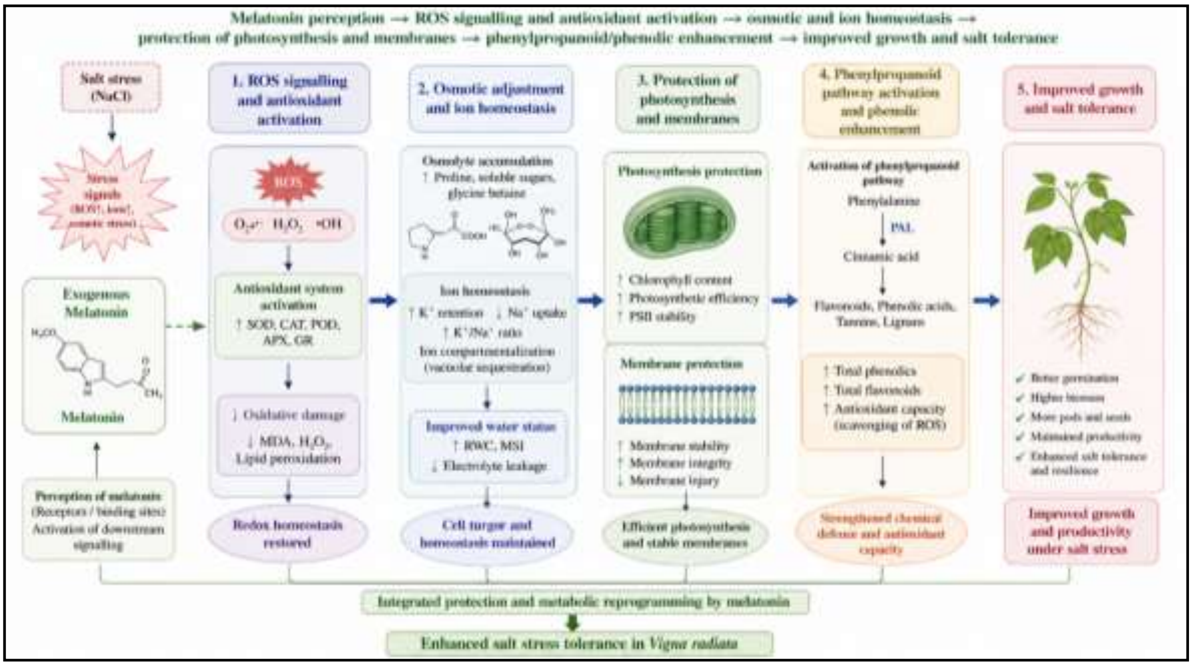

Figure 15. Proposed mechanistic model

5. CONCLUSION

The present study showed that there were significant salinity tolerance differences among the genotyped *Vigna radiata* with ML2056 comparatively more salinity tolerant and ML1808 comparatively less salinity tolerant. Germination, biomass accumulation, reproductive performance, photosynthetic pigments, relative water content, membrane stability were negatively affected while antioxidants and electrolyte leakage and markers of oxidative stress were accumulated by salinity. Some of these effects were substantially altered by exogenous melatonin, particularly considered as regards the content in Chlorophyll, the water status, the membrane status, activation of antioxidant defense system and lack of oxidant damages. Moreover, the concise increase in phenolics and flavonoids by both the stressors (salt and melatonin), indicates somehow involvement of the secondary metabolism in adapting to stress. Coordinated regulation of ROS homeostasis, osmotic adjustment, ion balance, photosynthetic protection, membrane stability and phenylpropanoid metabolism are indicated as an integrated physiological, biochemical and multivariate analysis suggests that this is the way in which melatonin induced salt tolerance works. The characterisation of contrasting genotypes can be used as a starting point for future, in-depth studies on the mechanism of salinity tolerance and screening of salinity tolerance for breeding in mungbean.

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