

# Study of Relative Antioxidant Potential of Induced Variants of *Catharanthus roseus* (L.) G. Don in Salt Stress Condition for the Effective Selection of Salt Tolerant Variant

Ashutosh Kumar Verma\*

## ABSTRACT

**Objectives:** *Catharanthus roseus* (L.) G. Don is an important plant documented for variety of medicinal uses and salt tolerant potential. During genetic improvement programme an attempt of induced mutagenesis was made for isolation of improved genotype of *C. roseus* var. Nirmal (CIMAP 0865) and nine EMS induced variants were isolated. Present study was made for the assessment of relative antioxidative potential of these variants and identification/selection of salt tolerant genotype. **Methods:** For the assessment of relative antioxidative potential under glass house condition one month old seedlings of all variants exposed to salt stress condition and the activities of antioxidant enzymes viz., superoxide dismutase, peroxidase and catalase were estimated in leaf tissue at 4<sup>th</sup> day and 8<sup>th</sup> day of NaCl supply while proline and total alkaloid contents were estimated at 8<sup>th</sup> day of NaCl supply. **Results:** In general, NaCl imposition causes increase in activity of earlier two enzymes, decrease in catalase activity and stimulatory effect on proline and total alkaloid content. Values for these parameters varied with type of variants. Variants V2, V3 and V7 exhibited higher activity of antioxidant enzymes and had high accumulation of proline and total alkaloids. **Conclusion:** The variants V2, V3 and V7, having higher estimates for these parameters than that of parental variety which indicates their better survival/adaptive potential against salt stress condition and in context of salt tolerant may be utilized in genetic improvement programme of *C. roseus*.

**Key words:** Antioxidant enzymes, Variants, *Catharanthus roseus*, Salt stress.

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## INTRODUCTION

Salinity in soil presents a stress condition for growth and development of the plants. Under natural conditions, plants are inevitably exposed to different types of stresses which may cause increased production of Reactive Oxygen Species (ROS) such as superoxide radical, H<sub>2</sub>O<sub>2</sub> and hydroxyl radical particularly in chloroplasts and mitochondria.<sup>1,2</sup> Generation of ROS causes rapid cell damage by triggering off a chain reaction.<sup>3</sup> Plants under stress produce some defense mechanisms to protect themselves from the harmful effect of oxidative stress. ROS scavenging is one of the common defense responses against abiotic stresses.<sup>4</sup> ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules like ascorbate and glutathione and enzymatic antioxidants.<sup>5</sup> The major ROS scavenging activities include complex non-enzymatic (ascorbate, glutathione, a-tocopherol) and enzymatic (Super oxide dismutase, Peroxidase and Catalase etc.) responses.<sup>6</sup> The pathways include the water-water cycle in chloroplasts and the ascorbate-glutathione cycle.<sup>7</sup> Antioxidant mechanisms may provide a strategy to enhance salt tolerance in plants. Among the several approaches to solve the problem

of saline soils, the biological approach to identify or grow salt-tolerant plants in such soils to enable soil reclamation is promising.<sup>8</sup> Considering the above mentioned facts, like crop plants it is also essential to test important medicinal plants for their salinity tolerance and the economic exploitation of saline soils.<sup>9</sup> *Catharanthus roseus* (L.) G. Don. is an important medicinal plant of the family *Apocynaceae* used for treating many fatal diseases and has good antioxidant potential but the salinity effect and antioxidant potential have attributed little attention.<sup>8</sup> Keeping this view an attempt of induced mutagenesis was made for the isolation/selection of improved genotypes of *C. roseus* var. Nirmal (CIMAP 0865) which have better salt tolerance potential and nine variants were isolated. This paper reports the findings on antioxidant potential of EMS induced variants of *C. roseus* under salt stress conditions.

## MATERIALS AND METHODS

One month old seedlings of nine EMS induced variants of *Catharanthus roseus* (L.) G. Don, var. Nirmal, (Table 1) were transplanted in refined sand at

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ambient temperature (15°-32°C) under glasshouse conditions in polythene containers (10") and supplied with the basal nutrient solution for about one month.<sup>10</sup> The composition of the basal nutrient solution was: 4 mM Ca(NO<sub>3</sub>)<sub>2</sub>; 4 mM KNO<sub>3</sub>; 1 mM Mg SO<sub>4</sub>; 1.5 mM NaH<sub>2</sub>PO<sub>4</sub>; 0.1 mM NaCl; 0.1m M Fe-EDTA; 10 µM MnSO<sub>4</sub>; 1.0 µM ZnSO<sub>4</sub>; 1.0 µM CuSO<sub>4</sub>; 30 µM H<sub>3</sub>BO<sub>3</sub>; 0.2 µM Na<sub>2</sub>MoO<sub>4</sub>; 0.1 µM CoSO<sub>4</sub> and 0.1 µM NiSO<sub>4</sub>. Thereafter, the plants were divided into two lots. One lot was allowed to grow with basal nutrient solution to serve as control while the second lot was grown in the nutrient solution having 200 mM NaCl. Deionized water was used throughout the culture period and contamination of chloride (Cl<sup>-</sup>) from any other resource was avoided and nutrient solution was supplied daily. The activities of antioxidant enzymes viz., Superoxide Dismutase (SOD), Peroxidase (POD) and Catalase (CAT) were estimated in leaf tissue at 4<sup>th</sup> day and 8<sup>th</sup> day of NaCl supply while proline and total alkaloid contents were estimated at 8<sup>th</sup> day of NaCl supply.

SOD, POD and CAT activities were measured by the method of, Beauchamp and Fridovich,<sup>11</sup> Luck method<sup>12</sup> and Euler and Josephson<sup>13</sup> method, respectively. For expressing the enzyme activity on protein basis, soluble protein content in enzyme extract was measured after precipitation with trichloroacetic acid by the method of Lowry *et al.*<sup>14</sup>

**Table 1: Different type of variants/mutants formed at different concentration of EMS treatment.**

Variant	Characters	Formed at
V1	Dwarf	0.50% EMS
V2	Base branching and high root alkaloid accumulating variant.	0.50% EMS
V3	Early flowering variant.	0.50% EMS
V4	Dwarf and low alkaloid accumulating variant.	0.75% EMS
V5	Base branching variant.	0.75% EMS
V6	Early flowering variant.	1.00% EMS
V7	Semi dwarf, seven petaled flower containing	0.75% EMS
V8	Early flowering, high leaf biomass yielding variant	1.00% EMS
V9	Early flowering	1.00% EMS

using bovine serum albumin (Sigma) as standard. Proline content was measured following the method of Bates *et al.*<sup>15</sup> Total alkaloid content (µgm/g) estimation in leaf tissue was done by the methodology developed by Narsasimhan *et al.*<sup>16</sup> All statistical analysis's were carried out by using STATICA version 6.0 software.

## RESULTS

Performance of ten genotypes (9 EMS induced variants + parent plant) of *C. roseus* var. Nirmal were assessed under salt stress condition by studying biochemical parameters viz. activity of SOD, POD, CAT, accumulation of proline and total alkaloid in leaf tissue. Data pertaining results of this experiment are summarized in Table 2, 3 and 4.

Results indicates that in general, imposition of NaCl stress caused an increase in SOD, POD activity while decrease in CAT activity. At day 4 maximum increase in SOD activity over respective control was noted for variant V2 (227%) and V7 (227%) while minimum for V-1 (0.90%) At day 8 it was noted maximum for V2 (203%) and minimum for variant V1 (11.2%). At day 4 maximum increase over respective control values for POD activity was found for V-3 (535%) and minimum for V9 (2.5 %) while at day 8 it was maximum for V-7 (486%) and minimum for V-9 (9.1%) (Figure 1 and 2).

Catalase activity in all genotypes except for V2, V3, V5 and V7 were decreased at day 4 of NaCl supply. This decrease was noted maximum for parent plant (- 61.8%) and minimum for V6 (-0.70%) at day 4 while at 8<sup>th</sup> day of NaCl supply decrease in CAT was found in all mentioned genotype except for parent, V2, V3 and V7. It was noted maximum for V9 (-32.6%) and minimum for V8 (-0.30%). Besides this, NaCl generated salt stress condition also showed stimulatory effect on proline and alkaloid biosynthesis. On 8<sup>th</sup> day of NaCl supply maximum increase over respective control values for total proline was found for variant V-7 (439%) and minimum for parent plant (5.83%) while total alkaloid content in leaf tissue was noted maximum for variant/ mutant V9 (69.1%) while minimum for V2 (12.3 %) respectively.

In this study significant increase in SOD activity over parent plant was noted for variant V<sub>2</sub>, V<sub>3</sub> and V<sub>7</sub> at 4<sup>th</sup> and 8<sup>th</sup> day of NaCl supply whereas, for V2 and V7 at day 4 and V9 at 8<sup>th</sup> day of NaCl supply. In general in our study sum up activity of both enzymes (SOD, POD) was noted higher in

**Table 2: Activity of antioxidative enzymes in *C. roseus* leaves in relation to excess NaCl supply at day 4.**

Treatments/ Variants	Parent	V1	V2	V3	V4	V5	V6	V7	V8	V9	LSD P=0.05
<b>Superoxide dismutase:</b>											
EU											
Control	14.5	28.9	11.5	15.6	15.4	14.7	18.2	13.4	22.6	29.9	14.3
NaCl	41.6	29.2	37.5	31.6	30.6	24.4	42.7	43.8	43.3	36.2	25.7
	(+188)	(+0.9)	(+227)	(+103)	(+98.7)	(+66.5)	(+134)	(+227)	(+91.9)	(+21.2)	
<b>Peroxidase: Difference in OD</b>											
Control	1.84	0.49	0.50	0.20	0.55	0.54	1.32	0.33	0.88	1.19	0.37
NaCl	1.92	1.75	1.14	1.27	1.56	0.84	1.36	1.10	0.96	1.22	0.56
	(+4.3)	(+257)	(+128)	(+535)	(+184)	(+48.2)	(+3.0)	(+233)	(+9.1)	(+2.5)	
<b>Catalase: µ moles H<sub>2</sub>O<sub>2</sub> decomposed</b>											
Control	1749	756	791	568	787	720	900	939	711	829	40.4
NaCl	668	709	937	977	740	750	894	816	654	659	36.2
	(-61.8)	(-6.3)	(+18.4)	(+71.9)	(-6.1)	(+4.2)	(-0.7)	(+13.1)	(-8.0)	(-20.4)	

Figures in parenthesis denote % increase or decrease over respective control

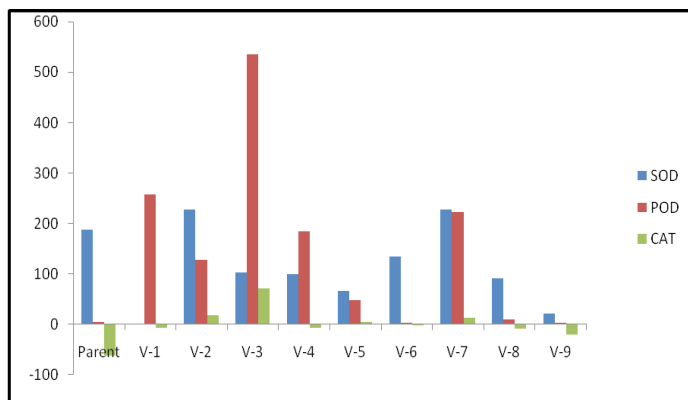


Figure 1: Percentage increase /decrease over respective control in different biochemical parameters after 4<sup>th</sup> day of treatment.

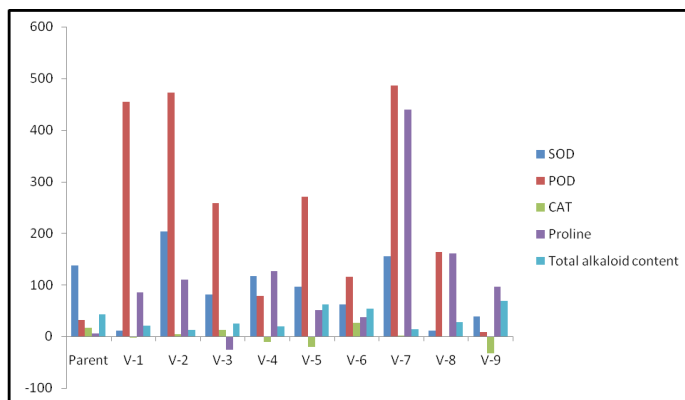


Figure 2: Percentage increase /decrease over respective control in different biochemical parameters after 8<sup>th</sup> day of treatment.

Table 3: Activity of antioxidative enzymes in *C. roseus* leaves in relation to excess NaCl supply at day 8.

Treatments/ Variants	Parent	V1	V2	V3	V4	V5	V6	V7	V8	V9	LSD P=0.05
<b>Superoxide dismutase: EU</b>											
Control	20.4	16.8	17.0	16.6	21.1	24.3	34.8	24.9	42.0	24.9	17.7
NaCl	48.6	18.6	51.4	29.7	45.7	50.8	56.6	63.5	46.8	34.5	23.7
	(+138)	(+11.2)	(+203)	(+81)	(+117)	(+96.8)	(+62.5)	(+155)	(+11.3)	(+38.7)	
<b>Peroxidase: Difference in OD</b>											
Control	0.64	0.34	0.56	0.53	1.18	0.63	0.83	0.83	1.18	1.76	0.52
NaCl	2.63	1.89	3.21	1.87	2.10	2.28	1.79	4.87	3.11	1.92	1.02
	(+31.1)	(+455)	(+473)	(+258)	(+78)	(+270)	(+116)	(+486)	(+164)	(+9.1)	
<b>Catalase: <math>\mu</math> moles H<sub>2</sub>O<sub>2</sub> decomposed</b>											
Control	1115	731	849	679	719	744	1008	943	919	724	51.5
NaCl	1307	709	883	765	639	694	742	960	916	488	68.4
	(+17.1)	(-2.6)	(+4.0)	(+12.5)	(-11.2)	(-20.2)	(-26.4)	(+1.9)	(-0.3)	(-32.6)	

Figures in parenthesis denote % increase or decrease over respective control

Table 4: Proline and total alkaloid in *C. roseus* leaves in relation to excess NaCl supply at day 8.

Treatments / Variants	Parent	V1	V2	V3	V4	V5	V6	V7	V8	V9	LSD P=0.05
<b>Proline: <math>\mu</math>g proline/100 mg fresh weight</b>											
Control	60.0	53.0	50.0	50.0	47.0	48.0	30.5	21.5	27.5	29.0	31.2
NaCl	63.5	72.0	105.5	63.0	106.0	72.0	42.0	116.0	72.0	57.0	26.0
	(+5.83)	(+84.8)	(+110)	(26.3)	(+126)	(+50.7)	(+37.7)	(+439)	(+161)	(+96.6)	
<b>Total alkaloid: <math>\mu</math>g/g dry weight</b>											
Control	74.0	84.0	100.0	80.0	72.0	73.0	60.0	87.5	85.0	84.0	52.2
NaCl	106.0	102.0	114.0	100.0	86.0	118.0	92.0	100.0	108.0	100.0	77.4
	(+43.2)	(+21.4)	(+12.3)	(+25)	(+19.4)	(+61.6)	(+53.3)	(+14.3)	(+27.1)	(+69.1)	

Figures in parenthesis denote % increase or decrease over respective control

variant V2, V3 and V7 (Table 2 and 3) which suggesting the existence of an effective scavenging mechanism to remove ROS.

Moreover, catalase activity was also found to be increased in variants V2, V3 and V7 with the salt application. Proline content was increased in the leaves of all variants with the salt supply which was found to be higher in variants V2 and V7 (Table 4).

## DISCUSSION

An increase in the activities of superoxide dismutase and peroxidase was found in all the variants supplied with 200 mM NaCl but it was more prominent in variants V2 and V7. Similar response to NaCl supply was reported in rice cultivars.<sup>17,18</sup>

The reduction in foliar SOD activity under high salinity can be also a consequence of an altered synthesis and accumulation of less active enzymes<sup>18</sup> and/or of a higher turnover of SODs.<sup>19</sup> The increase in POD activity under salt stress condition also observed in several plant species<sup>20</sup> and might be attributed to rapid diffusion of H<sub>2</sub>O<sub>2</sub> production in the cytosol or due to accumulation of high levels of phenols and reduced protein formation.<sup>21</sup> Hernandez *et al.*<sup>22</sup> reiterated that increase in the activities of SOD and POD was found more in the salt tolerant cultivars. Moreover, CAT activity was also increased in V2, V3 and V7 with the salt application. Similar results have earlier been reported in rice<sup>17</sup> and *Raphanus sativa*.<sup>23</sup> Higher catalase activity might help in protecting the membranes from lipid peroxidation due to destruction of H<sub>2</sub>O<sub>2</sub> which is a toxic metabolite produced by dismutation of superoxide radical by SOD. Proline content increased in leaves of all the variants with the salt supply which was found to be higher in variants V2 and V7. The enhancement in proline content under salt stress conditions has been reported to be a signal of water stress and the salt tolerance because proline can be considered as a compound which reduces the nitrogen and carbon skeleton for stress recovery.<sup>24</sup> According to Rossa-Iberra and Maiti<sup>25</sup> the increase in proline is probably due to the capacity of some plants to accumulate organic (sucrose, glucose) and inorganic (Na, K, Cl) metabolites in the cytoplasm to reduce the water potential and change in osmotic gradient, assuring the water flow to the plant and hereby the increased tolerance. NaCl supply also enhanced the alkaloid content in consonance with that of the report of Karadye and Gaikwad<sup>9</sup> in *C. roseus*.

## CONCLUSION

Although in our results variation in enzyme activity and other mentioned parameters were noted for all the variants but in conclusion the variants V2, V3 and V7 of *C. roseus* exhibiting higher activities of antioxidant enzymes with more proline content this indicates they contain better survival potential against salt stress condition because higher level of these enzymes minimize the effect of ROS generated as a effect of salt stress condition, are highly reactive in absence of any protective mechanism and they can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, pigments and nucleic acids due to which survival of plant is not possible. These variants will be used for developing the salt tolerant genotypes in *Catharanthus roseus*.

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## CONFLICT OF INTEREST

The author declares no conflict of interest.

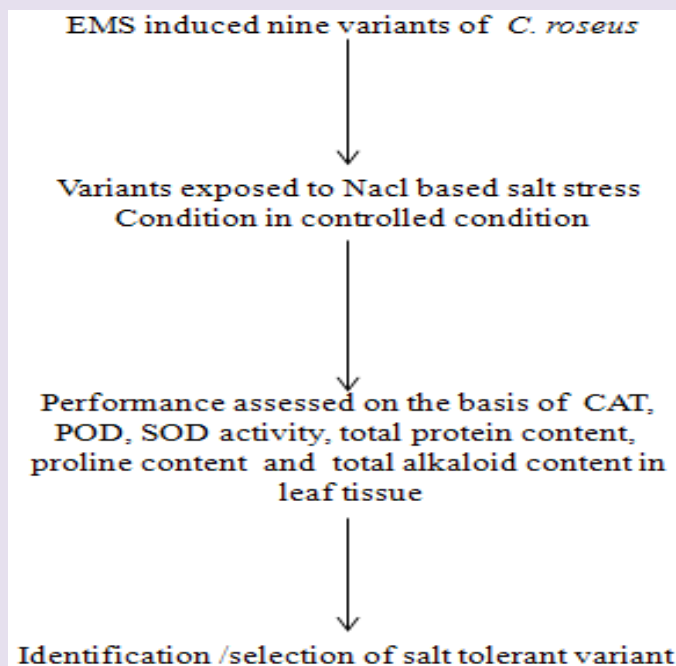
## ABBREVIATIONS

**ROS:** Reactive oxygen species; **SOD:** Superoxide dismutase; **POD:** Peroxidase; **CAT:** Catalase.

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### GRAPHICAL ABSTRACT



### SUMMARY

- Manuscript denotes scope of mutation breeding in the development of elite salt tolerant genotypes.
- Demonstrated salt tolerant nature of *C. roseus*.
- Selected genotypes may be used for soil reclamation and genetic improvement programmes of *C. roseus*.

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