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Salicylic Acid Induced Resistance against Mungbean Yellow Mosaic Virus (MYMV) and Enhanced Seed Yield in Resistant and Susceptible Urdbean [*Vigna mungo* (L.) Heper] Genotypes

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ABSTRACT

Background: Urdbean's low productivity is largely due to its susceptibility against whitefly-transmitted mungbean yellow mosaic virus (MYMV) disease. The effect of Salicylic acid (SA) on MYMV disease resistance and its impact on seed yield under field conditions on diverse genotypes is largely unknown. Therefore, in present investigation, we have analysed the effect of SA on induction of antioxidant enzymes leading to MYMV resistance and enhanced seed yield in urdbean genotypes.

Methods: Different concentrations of SA were sprayed on 3 week-old susceptible urdbean genotype (LBG 623) and induction of antioxidant enzymes was analysed. A pot experiment was conducted to see the effect of SA on initial induction of antioxidant enzymes maintained over long period of time in 39 urdbean genotypes. Under field conditions, the effect of SA treatment on MYMV disease resistance and seed yield was assessed.

Result: Rise in antioxidant enzyme production was observed in SA treated urdbean plants challenged with MYMV. The field experiment revealed that exogenous SA application significantly reduced MYMV incidence and increased seed yield in all 39 urdbean genotypes tested. The ability to confer MYMV resistance along with the increase in seed yield suggests the incorporation of SA in effective MYMV management strategies in urdbean.

Key words: Antioxidant enzymes, Mungbean yellow mosaic virus, Salicylic acid, Urdbean [*Vigna mungo* (L.) Heper].

INTRODUCTION

Urdbean [also known as black gram, *Vigna mungo* (L.) Heper] is an important pulse crop in South East Asia including India, Bangladesh, Pakistan, *etc.* (Kumari *et al.* 2020). The production and productivity of urdbean oscillates upon various environmental stresses. Among them, mungbean yellow mosaic virus (MYMV) causing yellow mosaic disease (YMD) is a most destructive disease of urdbean (Kumari *et al.* 2020). A plethora of reports estimates yield losses due to MYMV ranging from 10-100% depending upon susceptibility of urdbean genotypes, stage of crop infection and population of whitefly (*Bemisia tabaci*) (Nene 1972; Kumari *et al.* 2020). The MYMV infection also impairs the grain size and quality of urdbean (Kumari *et al.* 2020). Controlling this disease largely depends upon planting resistant genotypes and restricting whitefly population, a well-known natural transmitter of MYMV (Abubakar *et al.* 2018). Although several tolerant urdbean genotypes against MYMV have been identified (Saha *et al.* 2017; Kumari *et al.* 2020), but most of the tolerant genotypes are lacking yield potential under diverse environmental conditions. Further, breakdown of MYMV resistance is a problem associated with breeding for resistance in urdbean against MYMV due to the rapid formation of new pathotypes (Sehrawat *et al.* 2016; Saha *et al.* 2017).

Induction of Systemic acquired resistance (SAR) by exogenous application of salicylic acid (SA) is well established in plants (Umar *et al.* 2019). The role of SA in conferring MYMV resistance in mungbean (Umar *et al.* 2019) and urdbean were reported earlier (Kundu *et al.* 2011). Interestingly, application of SA was also linked with increase in seed yield in urdbean (Rawat *et al.* 2019). However, no systemic effort has been made to see the effect of SA in management of MYMV and seed yield in urdbean under field conditions. We chose to address these questions by

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analyzing the effect of SA on induction of defense related enzymes and further comparing its effect on MYMV resistance and grain yield in 39 urdbean genotypes. Overall, a net positive impact on MYMV tolerance and enhanced seed yield was achieved by exogenous application of SA in urdbean genotypes. These findings also provide a strong base for developing SA-based management programs for urdbean improvement.

MATERIALS AND METHODS

All the field experiments were conducted for two consecutive seasons during *Kharif,* 2018 and 2019 at Tirhut College of Agriculture (TCA), Dholi; Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India.

Activation of defense related enzymes

Three week-old healthy urdbean plants grown in earthen pot (inner dimension 20 × 27 sq. cm) were sprayed with 3 different concentrations of SA (50, 100 and 150 µM) along with a mock (sprayed with distilled water only). The induction of POD, SOD and CAT activities were observed at 24, 48 and 72 h after SA spraying. The experiments were laid out in a randomized block design (RBD) with six replications per treatment.

Furthermore, a pot experiment in RBD with six replications per treatment was performed to evaluate the effect of SA on initial induction of antioxidant enzymes maintained over a longer period of time. Three week-old healthy urdbean plants grown in earthen pots were sprayed with 100 µM SA, along with a mock treatment and transferred to an infected urdbean field with high MYMV disease pressure. After the first, second and third weeks of post-inoculation, the enzymatic activities of POD, SOD and CAT were determined in LBG 623. The enzyme activities of all the 39 urdbean genotypes were also estimated after two week of post-inoculation.

Antioxidant enzyme assay

POD enzyme activity was analyzed spectrophotometrically using guaiacol as substrate (Singhai *et al.* 2011). CAT activity was estimated spectrophotometrically by measuring the rate of H_2O_2 disappearance at 240 nm (Miyagawa *et al.* 2000). SOD activity was determined spectrophotometrically by measuring the inhibition of nitro-blue tetrazolium (Beauchamp *et al.* 1971). Total soluble protein was calculated using the method of Bradford (Bradford 1976). Enzyme specific activity was expressed as the number of enzyme units per milligram of protein.

Effect of SA on MYMV resistance in urdbean

The urdbean genotypes were tested for MYMV resistance under natural field conditions by planting two sets of experiments. In 1st set, foliar application of SA (100 µM) were applied on three week-old urdbean genotypes, whereas in 2nd set, plants were sprayed with distilled water only (mock). Both the experiments were laid out in RBD with two replications. Two rows of spreader (highly susceptible urdbean genotype, LBG 623) were planted all around the experiment in order to attract white fly and enhance infection of MYMV. All the cultural practices were adopted except for application of insecticide in order to encourage the population of whitefly for natural disease spreading.

MYMV incidence analysis

The urdbean genotypes were tested for MYMV resistance by visually assessing symptoms in the field and categorizing them from resistant to highly susceptible on a scale of 1 to 9 (Kumari *et al.* 2020). The per cent disease index (PDI) was calculated using Wheeler's formula at weekly intervals (1969).

$$PDI = \frac{\text{Sum of all the numerical ratings}}{\text{Number of observations} \times \text{Maximum disease rating}} \times 100$$

Seed yield

Ripened pods from each genotype were hand-picked, allowed to dry for 10 days, then separated and weighed to determine yield. The mean data for seed yield in both years and their pooled data were subjected to analysis of variance for RBD.

Data analysis

All the experiments were repeated twice and pooled data were analysed with SPSS and ANOVA. The least significant difference (LSD) was used to compare treatment means using a multiple mean-comparison test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The wide host range of the MYMV and whitefly vectors makes managing this notorious disease very challenging (Varma *et al.* 1998). The use of SA, a known inducer of SAR in plant, has recently been employed in controlling MYMV *via* induction of antioxidant enzymes (Umar *et al.* 2019). However, the effect of SA in controlling MYMV and seed yield in urdbean under field conditions are less explored. The need for improved management strategy against MYMV in urdbean encouraged us to investigate the role of SA in the induction of antioxidant enzymes *viz.* POD, SOD and CAT leading to enhanc disease resistance and higher seed yield in urdbean.

Activation of defense related enzymes

To examine the effect of SA on the induction of defense-related enzymes, three week-old healthy urdbean plants were sprayed with three different concentrations of SA (50, 100 and 150 M), as well as a mock treatment. The gradual increase in the activities of all the three enzymes were observed as the concentration of SA treatment was increased in all times point (24, 48 and 72 h after treatment) studied (Fig 1). Although the maximum enzymatic activities were observed at 150 µM SA treatment but the difference in enzymatic activities observed at 100 and 150 µM SA treatments were not significant (Fig 1). However, significant increase in enzymatic activities was recorded either 100 µM

or 150 µM over 50 µM of SA treatment (Fig 1). The exogenous application of SA induced the activity of antioxidant enzymes *viz*. POD, SOD, CAT and PAL in mungbean supports our study (Umar *et al.* 2019; Ali and Mahmoud, 2013).

Three week-old pot grown urdbean plants (LBG 623), sprayed with 100 M SA along with the mock, were transferred to MYMV infected urdbean field with high MYMV disease pressure to evaluate the effect of SA on initial induction of defence related enzymes maintained over a longer period of time. There was a ~3-fold increase in the production of antioxidant enzymes in SA treated urdbean plants challenged with MYMV compared to mock treated plants (Fig 2). However, no significant differences in the enzymatic activities of POD, CAT, and SOD were observed at 1st, 2nd and 3rd week post treatment (Fig 2). Further, enzymatic activities were estimated in all the 39 urdbean genotypes (SA and mock treated) at 2 week post-inoculation of MYMV. The POD activities in SA treatment followed by MYMV infection showed more than 4-fold rise in disease free or highly resistant genotypes, while ~3-fold increase in susceptible urdbean genotypes were observed (Fig 3). The resistant or moderately resistant genotypes showed up to 4 fold increase in POD activities (Fig 3). SA treatment followed by MYMV infection increase CAT activities by ~4-fold, ~3.5, and ~3-fold in disease free or highly resistant, moderately resistant and susceptible urdbean genotypes, respectively (Fig 4). Similarly, the SOD showed more than 3.5-fold increase in disease free or highly resistant genotypes, while ~2-fold rise in susceptible urdbean genotypes were observed (Fig 5). The resistant or moderately resistant genotypes showed up to 3.5-fold increase in SOD activities (Fig 5). Taken together, greater induction in POD, CAT and SOD activities were observed in genotypes belonging to resistant group.

The induction of isoforms of SOD and POD in urdbean leaves upon SA treatment supports our results (Kundu *et al.* 2011). The high level of antioxidant enzymes produced due to the application of SA before the pathogen challenge led the plant to induce defence responses comfortably and effectively (Umar *et al.* 2019). The maintenance of a high

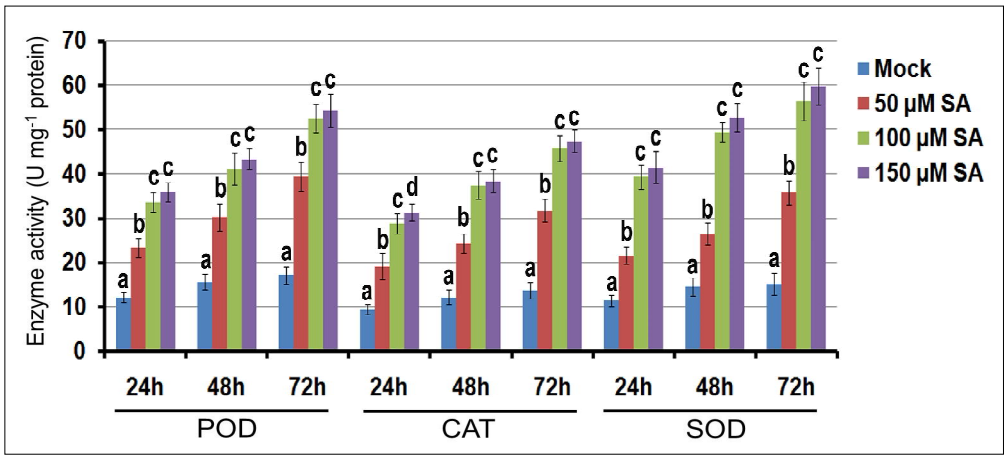

Fig 1: Antioxidant enzyme activity in LBG 623 in response to SA treatment. POD, CAT and SOD enzyme activities were analyzed upon different concentration of SA treatment at 24, 48 and 72 h.

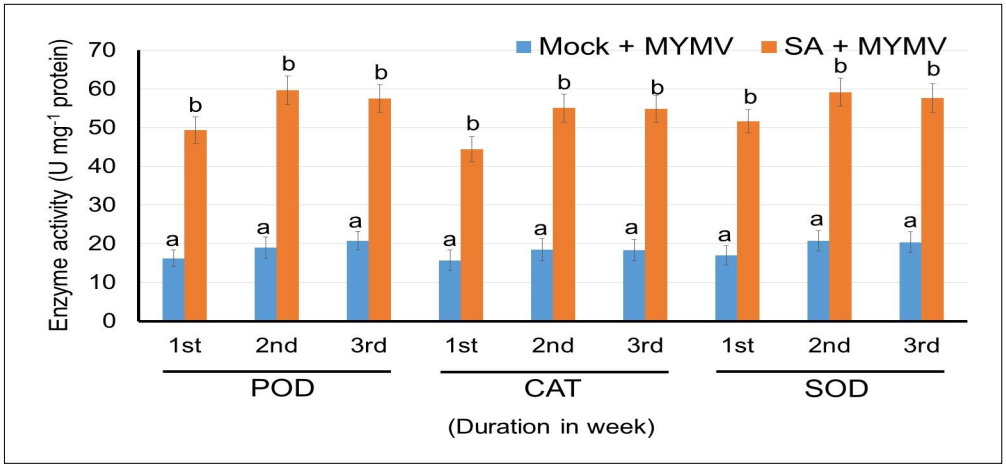

Fig 2: Antioxidant enzyme activity in LBG 623 in response to SA and MYMV treatment. POD, CAT and SOD enzyme activities were analyzed after exogenous application of SA followed by MYMV treatment for 1st, 2nd and 3rd week.

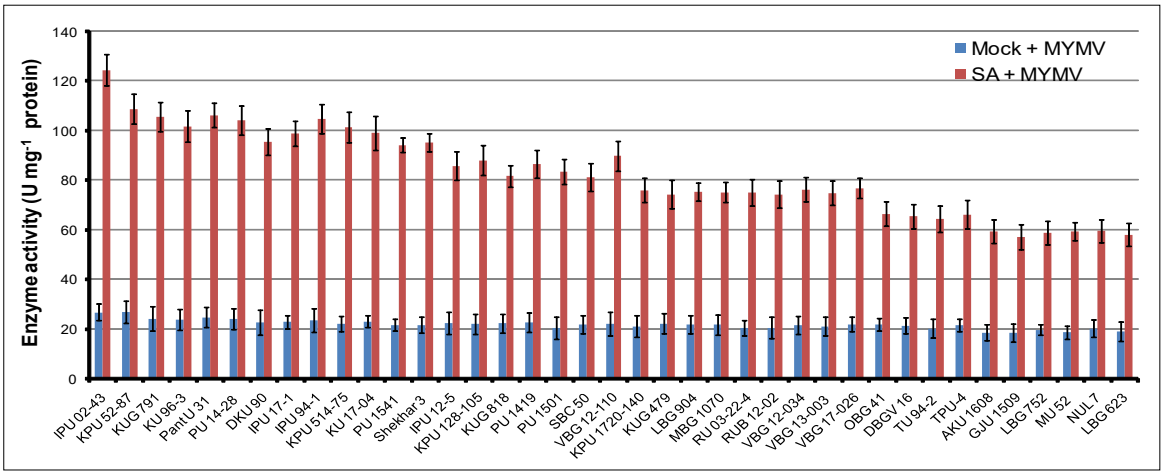

Fig 3: POD activity in urdbean genotypes in response to SA and MYMV treatment. POD enzyme activities were analyzed after exogenous application of SA followed by MYMV treatment for 2 week.

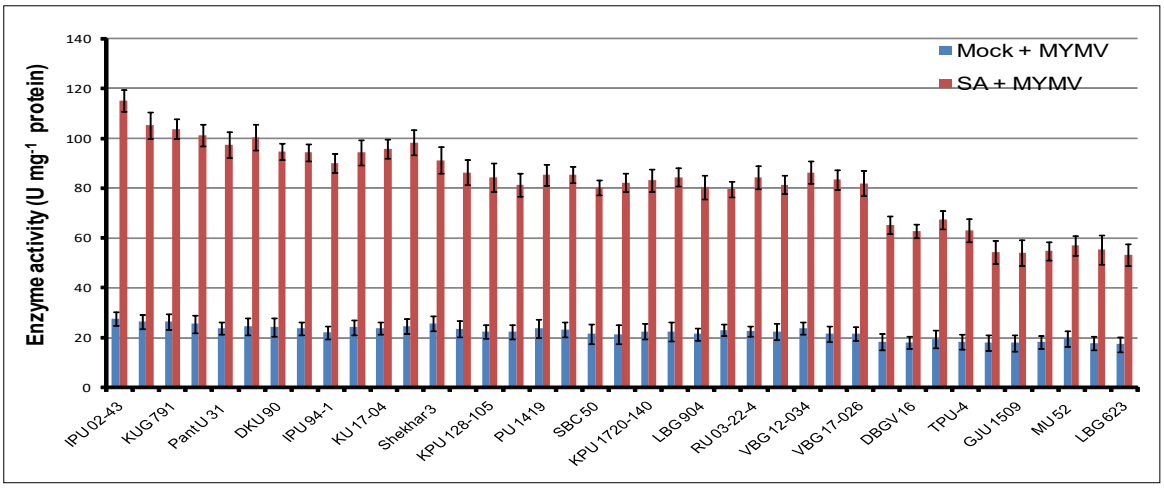

Fig 4: CAT activity in urdbean genotypes in response to SA and MYMV treatment. CAT enzyme activities were analyzed after exogenous application of SA followed by MYMV treatment for 2 week.

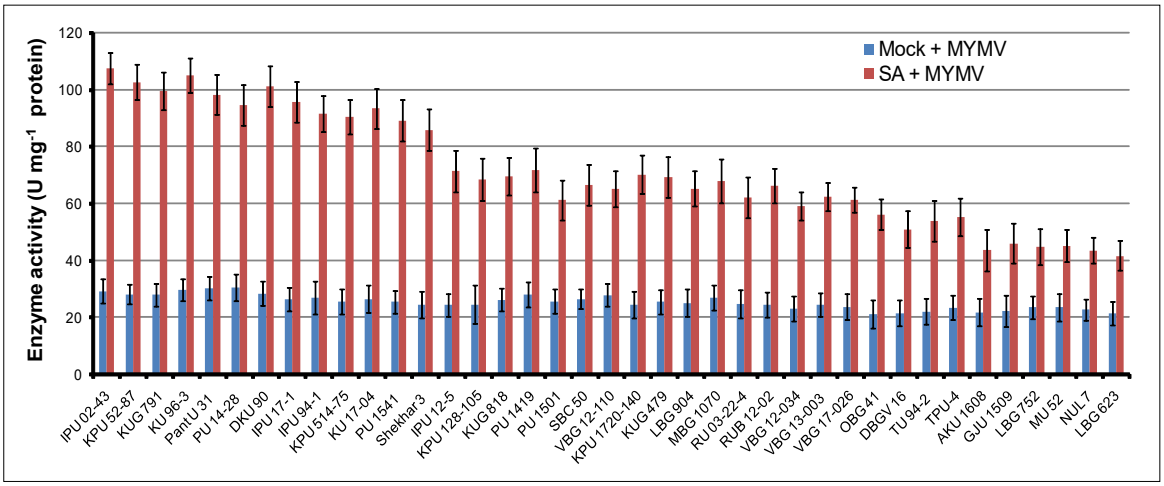

Fig 5: SOD activity in urdbean genotypes in response to SA and MYMV treatment. SOD enzyme activities were analyzed after exogenous application of SA followed by MYMV treatment for 2 week.

level of antioxidant enzymes for several weeks upon SA treatment was also supported by previous study on mungbean (Umar *et al.* 2019).

Effect of SA on MYMV resistance in urdbean

We have evaluated the effect of SA on MYMV resistance in urdbean genotypes at field conditions. At pod formation stage disease severity in 39 urdbean genotypes were analysed and grouped into different reactions group (Table 1). In control set, only six genotypes were found to be either disease free or highly resistance (HR), whereas 20 genotypes in SA treated set were observed to be either disease free or HR in both the year tested (Table 1). In control set, 6 genotypes showed highly susceptible (HS) reaction in both the year tested, whereas none of the genotypes showed HS reaction against MYMV (Table 1). These results clearly demonstrate the profound effect of SA treatment on urdbean genotypes against MYMV. The induced resistance was observed in all the genotypes irrespective of tolerant or susceptible genotypes. Therefore the protection observed might be due to the induction of SAR. Our findings were duly supported by previous studies on the effect of SA in treatment on different mosaic viruses (Kundu *et al.* 2011; Farooq *et al.* 2018). The mechanisms by which SA promotes tolerance against MYMV may consist of higher basal defense preparedness, as indicated by the early induction of antioxidant enzymes. Previous studies showed that SA facilitates production of antioxidant enzymes, which induced SAR mediated disease resistance against several viral diseases (Elbadry *et al.* 2006; Siddique *et al.* 2014) including MYMV in mungbean and urdbean (Umar *et al.* 2019; Kundu *et al.* 2011). Taken together, exogenous application of SA leads to the activation of antioxidant enzymes, which persist for a long period and provide effective protection against

Table 1: Reaction of urdbean genotypes against MYMV during *Kharif* 2018 and 2019.

Genotypes	Genotypes reaction group	
	Untreated	SA treatment
IPU 02-43, KPU 52-87, KUG 791, KU 96-3, Pant U 31 and PU 14-28	Free-HR, HR-Free (0-5%)	Free-HR, HR-Free (0-3%)
DKU 90, IPU 17-1, IPU 94-1, KPU 514-75, KU 17-04, PU 1541 and Shekhar 3	HR-HR (2-5%)	Free-HR, HR-Free (0-5%)
IPU 12-5, KPU 128-105, KUG 818, PU 1419, PU 1501, SBC 50 and VBG 12-110	HR-R, R-HR, R-R (6-10%)	Free-HR, HR-Free (0-5%)
KPU 1720-140, KUG 479, LBG 904, MBG 1070, RU 03-22-4, RUB 12-02, VBG 12-034, VBG 13-003 and VBG 17-026	R-MR, MR-R (11-15%)	HR-HR (1-5%)
OBG 41, DBGV 16, TU 94-2 and TPU-4	HS/MS/S in one of season (20-50%)	R-R (6-10%)
AKU 1608, GJU 1509, LBG 623, LBG 752, MU 52 and NUL 7	HS-HS (50-75%)	MR (15-20%)

The data in parentheses indicate PDI on MYMV infected urdbean genotypes.

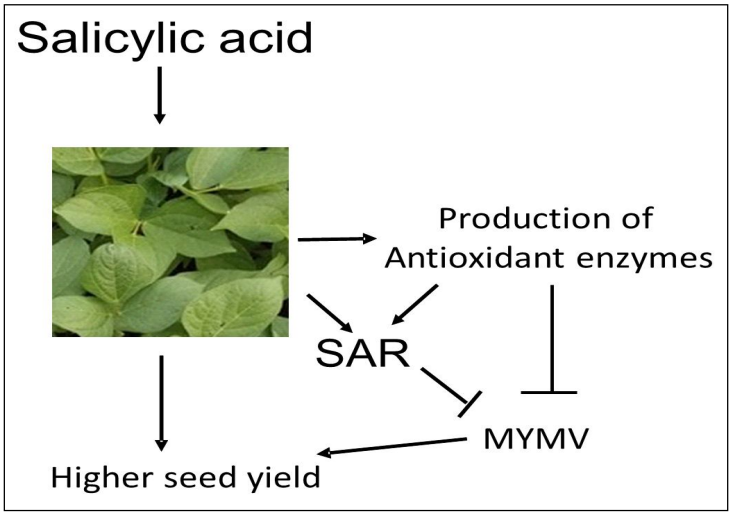

Fig 6: Model depicting SA-mediated increase in seed yield and MYMV tolerance in urdbean. The foliar application of SA enhanced the production of antioxidant enzymes, which induced SAR and inhibit MYMV infection. The inhibition of MYMV and direct beneficial effect of SA showed increased seed yield in urdbean plants.

MYMV in urdbean. The enhanced resistance observed in this study could be due to synergistic effect of SA mediated induction of SAR and antioxidant enzymes.

Effect of SA on seed yield of urdbean

SA is known for its pleiotropic effect on plants including crop yield (Liu *et al.* 2015; Ali and Mahmoud 2013). Therefore, we tempted to analyse the increase in seed yield of urdbean genotypes on SA treatment under field conditions. The yield analysis showed that there were increase in the yield of all SA treated urdbean genotypes (Table 2). The increase in yield was ranging from 23.98 to 31.97% (Table 2). The maximum increase in yield was observed in DKU 90 followed by IPU 12-5 and AKU 1608. A plethora of reports indicate that foliar application of SA had a beneficial effect on growth and photosynthesis in crop plants, leading to an increase in seed yield in normal as well as under stress conditions (Ali and Mahmoud 2013; Rawat *et al.* 2019).

Table 2: Yield analysis of urdbean genotypes upon SA treatment.

Genotypes	Seed yield* (Kg/ha)	
	Untreated	SA treatment
AKU 1608	1057.25	1377.28
DKU 90	1078.47	1423.29
GJU 1509	781.12	1002.47
IPU 02-43	916.58	1136.42
IPU 12-5	889.72	1165.36
IPU 17-1	957.42	1233.54
IPU 94-1	960.54	1225.81
KPU 128-105	996.36	1251.06
KPU 1720-140	942.45	1180.98
KPU 514-75	1162.36	1480.11
KPU 52-87	1147.76	1439.28
KU 17-04	833.62	1070.66
KUG 479	860.24	1085.67
KUG 791	848.28	1058.56
KUG 818	974.05	1253.41
KU 96-3	1017.16	1279.2
LBG 752	928.72	1176.92
LBG 904	1028.52	1293.5
MBG 1070	980.16	1254.39
MU 52	1083.52	1401.75
NUL 7	1044.65	1340.58
OBG 41	1258.27	1610.87
DBGV 16	991.35	1277.97
Pant U 31	942.15	1175.14
PU 1419	959.41	1197.48
PU 14-28	992.62	1231.34
PU 1501	1017.42	1313.64
PU 1541	1009.05	1293.82
RU 03-22-4	1048.26	1329.69
RUB 12-02	901.49	1142.05
SBC 50	1018.05	1279.2
Shekhar 3	990.42	1258.64
TU 94-2	941.24	1180.4
VBG 12-034	1029.48	1318.01
VBG 12-110	1216.52	1537.9
VBG 13-003	1112.54	1424.75
VBG 17-026	1225.26	1549.6
TPU-4	956.37	1199.9
LBG 623 (C)	876.27	1125.4
CD at 5%	173.0	250.4
SEM	61.43	88.91
C.V. (%)	10.64	12.12

*Data are mean value of 2 seasons.

Based on the results obtained in this study, a model incorporating MYMV resistance couple with increased seed yield mediated directly by SA or *via* induction of antioxidant enzymes in urdbean are depicted in Fig 6.

CONCLUSION

In this study, we found that exogenous application of SA causes an initial induction of antioxidant enzymes that is sustained over time, which aids in the management of MYMV disease. The field experiment revealed that exogenous SA application significantly reduced MYMV incidence and increased seed yield in urdbean genotypes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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