CHAPTER 7

PROTEIN, AMINO ACID AND ORGANIC ACID ESTIMATION

7.1 Protein estimation

In 10 days old control seedlings, protein content observed was 8.9 mg g^{-1} fr. wt. which was decreased to 5.7 mg g^{-1} fr. wt. in the seedlings grown in Petri-plates supplemented with 250 mg IMI L^{-1}. But, increase in protein content was observed to 8.5 mg g^{-1} fr. wt. in seedlings raised from 100 nM EBR treated seeds and grown in 250 mg IMI L^{-1} solutions in Petri-plates (Table 7.1.1, Fig. 7.1.1).

Table 7.1.1 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on protein content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Protein content (mg g^{-1} fr. wt.)		
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	8.9±0.9		
0	0.1	9.5±0.6		
0	1	10.1±1.6		
0	100	10.4±0.3		
150	0	7.8±0.6		
150	0.1	8.0±0.3		
150	1	10.4±0.4		
150	100	11.7±0.4		
200	0	7.4±0.6		
200	0.1	7.6±0.9		
200	1	8.3±0.5		
200	100	10.8±0.3		
250	0	5.7±0.2		
250	0.1	6.6±0.8		
250	1	6.8±0.5		
250	100	8.5±0.7		
Two-way ANOVA				
F_{IMI}		48.88***		
F_{EBR}		40.96***		
$F_{IMI \times EBR}$		2.58*		
HSD		1.15		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Protein = 9.54 - 0.0098 IMI + 0.0227 EBR		- 0.5569	0.5988	0.8178***
* and *** indicate significant at p<0.05 and p<0.001 respectively. r = multiple correlation coefficient.				

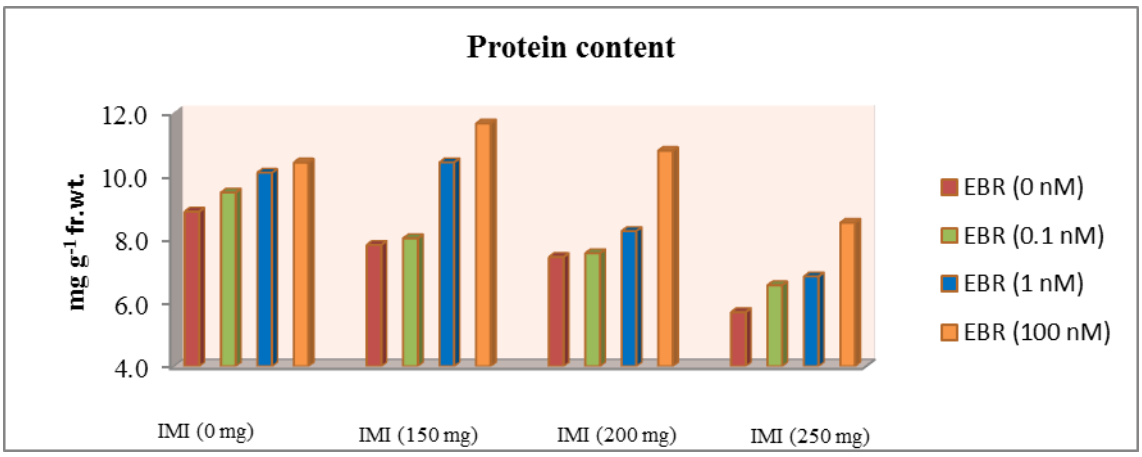

Fig. 7.1.1 Effect of seed soaking with EBR on protein content in *B. juncea* seedlings grown under IMI toxicity.

In the leaves of 30 days old *B. juncea* plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil), maximum decrease in protein content observed was 7.8 mg g^{-1} fr. wt. as compared to 9.9 mg g^{-1} fr. wt. in the control. In plants raised from 100 nM EBR treated seeds and grown under IMI toxicity, protein content was enhanced to 10.1 mg g^{-1} fr. wt. (Table 7.1.2, Fig. 7.1.2).

Table 7.1.2 Effect of seed soaking with 24-epibrassinolide (EBR) on protein content in *Brassica juncea* plants grown under imidacloprid (IMI) toxicity. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Protein content (mg g^{-1} fr. wt.)				
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS (Leaves)	60 DAS (Leaves)	65 DAS (Leaves)	80 DAS (Green pods)	90 DAS (Leaves)
0	0	9.9±0.8	13.2±0.7	12.1±1.1	5.2±0.4	5.7±1.1
0	0.1	9.7±0.8	13.8±0.7	12.5±1.2	5.3±0.7	6.7±0.4
0	1	10.7±1.0	14.2±1.3	13.3±2.8	5.9±0.9	6.8±0.6
0	100	10.8±0.5	15.3±0.7	13.9±0.7	6.3±0.9	7.4±0.2
250	0	9.4±0.3	10.1±0.4	10.1±0.8	4.9±0.1	5.2±0.2
250	0.1	9.9±0.3	10.4±0.6	10.4±2.0	5.4±0.3	5.5±0.3
250	1	10.2±0.5	11.4±0.7	10.9±0.5	5.9±0.3	6.0±0.3
250	100	11.0±0.7	13.2±0.9	12.2±0.8	6.9±1.1	7.3±0.2
300	0	9.2±0.8	9.2±0.4	8.2±1.0	4.3±0.5	4.5±0.4
300	0.1	9.9±1.3	10.3±0.9	8.7±0.6	4.8±0.6	5.0±0.5
300	1	10.3±0.9	10.2±0.9	10.2±1.2	5.2±0.6	5.0±0.5
300	100	11.2±0.2	14.0±0.7	13.4±1.1	5.7±0.5	5.7±0.5
350	0	7.8±0.4	8.2±0.05	6.2±0.4	3.2±0.2	3.9±0.2
350	0.1	8.8±1.0	9.0±1.2	7.1±0.7	4.1±0.3	4.2±0.1
350	1	9.1±1.2	9.1±1.3	9.2±0.6	4.1±0.1	4.3±0.2
350	100	10.1±0.8	11.9±0.5	10.7±1.6	4.8±0.2	5.1±0.4
Two-way ANOVA						

Treatments		Protein content (mg g^{-1} fr. wt.)				
IMI (mg Kg^{-1})	**EBR (nM L^{-1})**	**30 DAS (Leaves)**	**60 DAS (Leaves)**	**65 DAS (Leaves)**	**80 DAS (Green pods)**	**90 DAS (Leaves)**
F_{IMI}		7.33***	66.19***	27.82***	23.01***	59.55***
F_{EBR}		10.14***	39.57***	17.21***	15.62***	24.45***
$F_{IMI \times EBR}$		0.37	1.48	1.17	0.31	1.17
HSD		1.32	1.36	3.83	1.77	0.75

Multiple linear regression

MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Protein (30 DAS) = 10.11 - 0.0024 IMI + 0.0124 EBR	- 0.3815	0.6197	0.7277**
Protein (60 DAS) = 13.47 - 0.0121 IMI + 0.0286 EBR	- 0.7622	0.5746	0.9547***
Protein (65 DAS) = 12.56 - 0.0116 IMI + 0.0268 EBR	- 0.7102	0.5233	0.8822***
Protein (80 DAS) = 5.67 - 0.0036 IMI + 0.0114 EBR	- 0.5209	0.5323	0.7449**
Protein (90 DAS) = 6.53 - 0.0057 IMI + 0.0115 EBR	- 0.7400	0.4705	0.8769***

** and *** indicate significant at p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

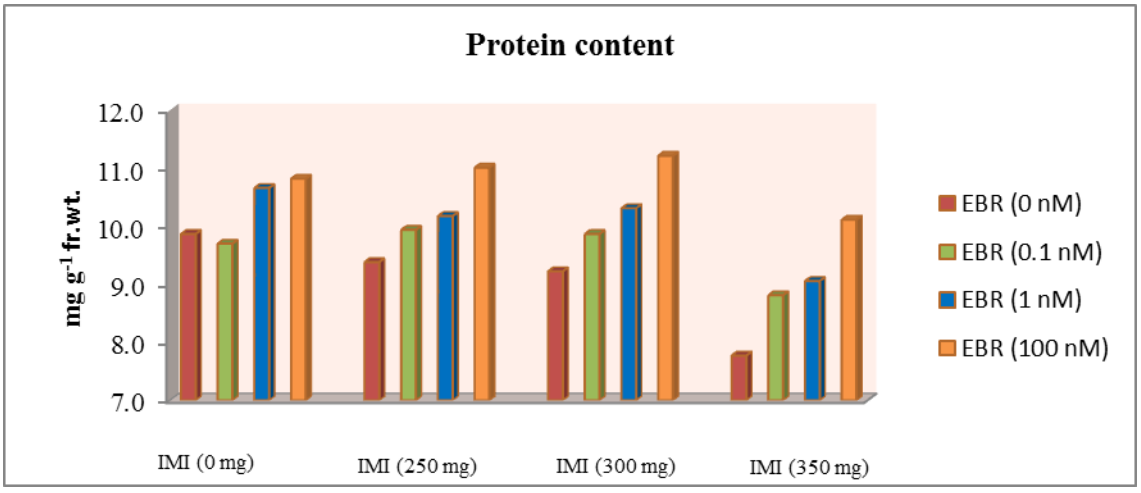

Fig. 7.1.2 Effect of seed soaking with EBR on protein content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Protein content observed in the leaves of 60 days old plants (Control) was 13.2 mg g^{-1} fr. wt. which was decreased to 8.2 mg g^{-1} fr. wt. in plants grown in soils containing 350 mg IMI Kg^{-1} soil. Seed soaking with EBR (100 nM) resulted in enhancement of protein content to 11.9 mg g^{-1} fr. wt. in plants grown under IMI toxicity (Table 7.1.2, Fig. 7.1.3).

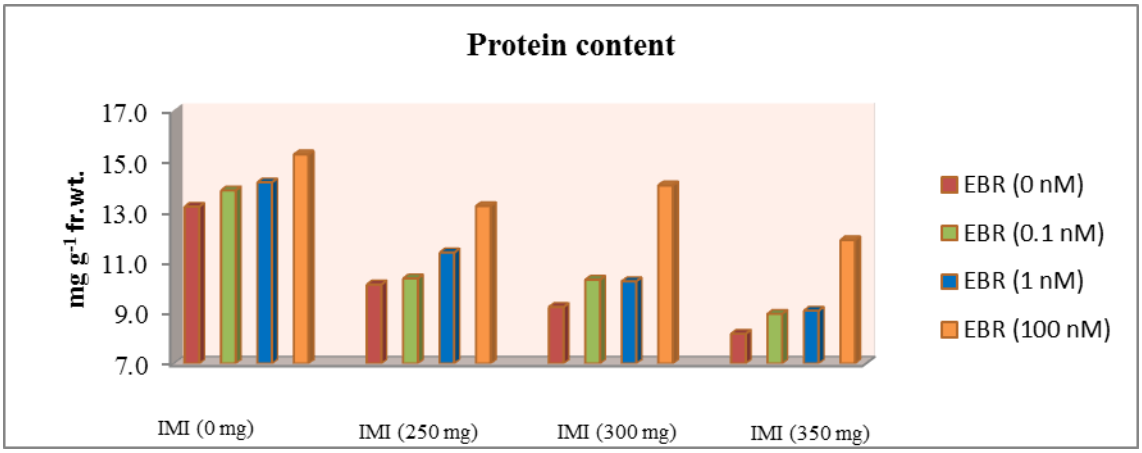

Fig. 7.1.3 Effect of seed soaking with EBR on protein content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In the leaves of 65 days old plants grown in IMI amended soils (350 mg IMI Kg^{-1} soil), protein content was decreased to 6.2 mg g^{-1} fr. wt. when compared to protein content in control plants (12.1 mg g^{-1} fr. wt.). But, increase in protein content to 10.7 mg g^{-1} fr. wt. was observed in plants raised from seeds pre-soaked with EBR (100 nM) and grown in pots containing 350 mg IMI Kg^{-1} soil (Table 7.1.2, Fig. 7.1.4).

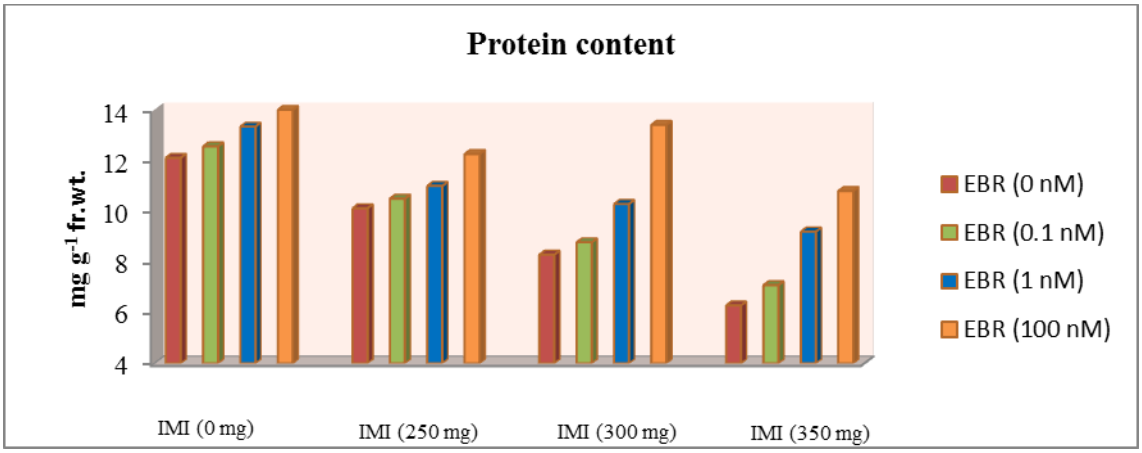

Fig. 7.1.4 Effect of seed soaking with EBR on protein content in the leaves of *B. juncea* plants (65 DAS) grown under IMI toxicity.

Protein content observed in the green pods of 80 days old control plants was 5.2 mg g^{-1} fr. wt., which was reduced to 3.2 mg g^{-1} fr. wt. in plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil). Increase in the protein content to 4.8 mg g^{-1} fr. wt. was observed in the green pods of plants germinated from EBR (100 nM) treated seeds and grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 7.1.2, Fig. 7.1.5).

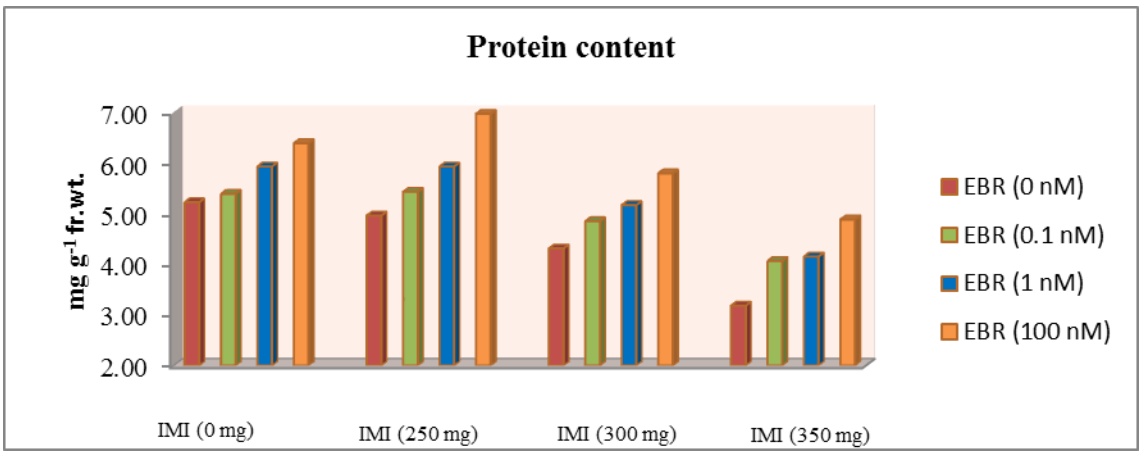

Fig. 7.1.5 Effect of seed soaking with EBR on protein content in the green pods of *B. juncea* plants (80 DAS) grown under IMI toxicity.

In the leaves of 90 days old control plants, protein content observed was 5.7 mg g^{-1} fr. wt., which was reduced to 3.9 mg g^{-1} fr. wt. in plants grown in pots containing 350 mg IMI Kg^{-1} soil. Increase in protein content to 5.1 mg g^{-1} fr. wt. was observed in plants raised from EBR (100 nM) treated seeds and grown under IMI stress (Table 7.1.2, Fig. 7.1.6).

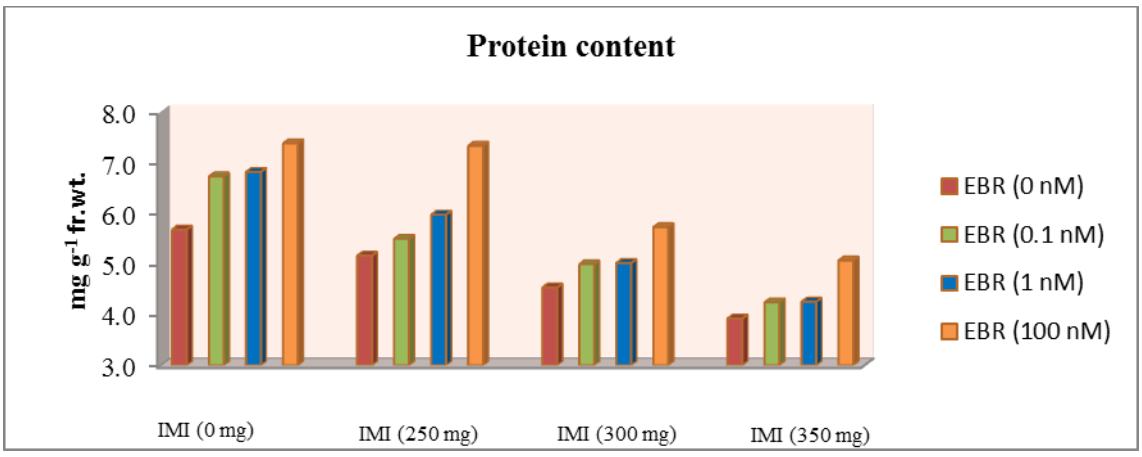

Fig. 7.1.6 Effect of seed soaking with EBR on protein content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Data analysis using two-way ANOVA and Tukey's HSD showed significant difference for protein contents in *B. juncea* plants. MLR analysis revealed that IMI application resulted in reduction of protein content (negative β_{IMI} values), whereas seed soaking in EBR before sowing resulted in increase of protein content as shown by positive β_{EBR} values (Tables 7.1.1 and 7.1.2).

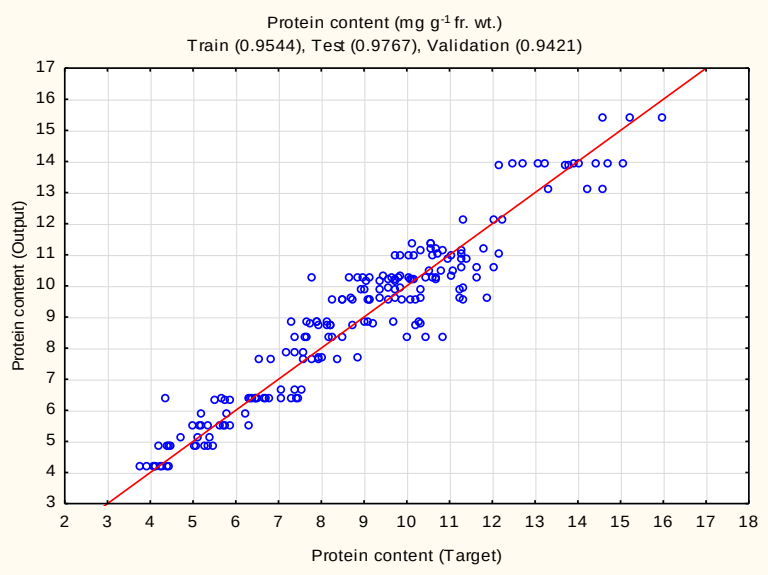

Fig. 7.1.7 Correlation between target (experimental) and output (simulated) protein contents using ANN model (p<0.001).

7.2 Amino acid analysis

In the seedlings and leaves of *B. juncea* plants, a total of 21 amino acids were detected. Content of total amino acids was observed to decrease with the application of IMI, whereas seed treatment with EBR resulted in increase of total amino acid content in plants grown under IMI toxicity.

In 10 days old control seedlings, total amino acid content was 9.59 mg g^{-1} fr. wt. which was decreased to 6.01 mg g^{-1} fr. wt. in seedlings grown in Petri-plates containing IMI solutions. Seed soaking with EBR before germination in Petri-plates containing IMI resulted in increase in total amino acid content to 9.05 mg g^{-1} fr. wt. (Table 7.2.1, Fig. 7.2.1).

Table 7.2.1 Effect of seed soaking with 24-epibrassinolide (EBR) on the contents of amino acids in 10 days old *Brassica juncea* seedlings grown under imidacloprid (IMI) toxicity. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Name	Treatments				F-ratios and HSD			
IMI (mg L^{-1})	0	0	200	200				
EBR (nM L^{-1})	0	100	0	100				
Amino acid content (mg g^{-1} fr. wt.)					F_{IMI}	F_{EBR}	$F_{IMI\times EBR}$	HSD
Aspartate	0.118±0.014	0.146±0.030	0.075±0.013	0.101±0.010	16.91**	6.44*	0.01	0.05
Glutamate	0.164±0.022	0.169±0.022	0.115±0.023	0.171±0.037	2.28	3.77	2.55	0.07
Asparagine	1.193±0.022	1.223±0.065	0.633±0.098	1.418±0.326	3.31	16.53**	14.17**	0.45
Serine	0.427±0.040	0.445±0.051	0.331±0.027	0.382±0.053	9.67*	1.77	0.41	0.11
Glutamine	1.085±0.064	1.266±0.028	0.729±0.078	0.893±0.191	33.69***	7.53*	0.02	0.28
Histidine	2.425±0.190	2.496±0.355	1.179±0.040	2.220±0.296	27.69***	14.79**	11.24*	0.65
Glycine	0.424±0.062	0.372±0.037	0.306±0.067	0.415±0.053	1.34	0.76	6.32*	0.15
Arginine	1.520±0.068	1.671±0.387	0.761±0.028	1.264±0.138	23.39**	7.36*	2.12	0.55

Name	Treatments				F-ratios and HSD			
IMI (mg L^{-1})	0	0	200	200				
EBR (nM L^{-1})	0	100	0	100				
Amino acid content (mg g^{-1} fr. wt.)					F_{IMI}	F_{EBR}	$F_{IMI \times EBR}$	HSD
Threonine	0.111±0.021	0.120±0.012	0.096±0.022	0.102±0.031	1.66	0.32	0.02	0.06
Alanine	0.556±0.109	0.512±0.078	0.351±0.049	0.441±0.055	9.77*	0.27	2.29	0.20
GABA	0.021±0.007	0.019±0.004	0.022±0.004	0.017±0.003	0.0007	1.53	0.22	0.01
Tyrosine	0.255±0.038	0.293±0.041	0.245±0.036	0.244±0.052	1.46	0.58	0.65	0.11
Cysteine	0.156±0.029	0.161±0.027	0.112±0.021	0.157±0.034	2.15	2.39	1.46	0.07
Valine	0.060±0.007	0.064±0.016	0.055±0.009	0.053±0.010	1.64	0.02	0.24	0.03
Methionine	0.117±0.015	0.122±0.016	0.090±0.022	0.109±0.014	3.97	1.61	0.50	0.04
Tryptophan	0.151±0.033	0.203±0.053	0.137±0.007	0.146±0.015	3.65	2.64	1.28	0.08
Phenylalanine	0.155±0.020	0.152±0.043	0.130±0.014	0.145±0.033	0.88	0.12	0.27	0.08
Isoleucine	0.128±0.017	0.175±0.034	0.106±0.017	0.125±0.023	6.89*	5.67*	1.07	0.06
Leucine	0.113±0.023	0.118±0.007	0.105±0.025	0.118±0.029	0.09	0.47	0.09	0.06
Lysine	0.133±0.030	0.152±0.018	0.117±0.030	0.122±0.027	2.10	0.61	0.19	0.07
Proline	0.281±0.055	0.349±0.058	0.319±0.048	0.409±0.083	1.84	4.86	0.08	0.16
TOTAL	**9.592±0.166**	**10.230±0.786**	**6.013±0.180**	**9.052±0.512**	**72.14*** **	**43.10*** **	**18.39****	**1.27**

Multiple linear regression

MLR equation	β-regression coefficients		Multiple correlation coefficient
	β_{IMI}	β_{EBR}	
Aspartate = 0.11 - 0.0002 IMI + 0.0003 EBR	- 0.7343	0.4532	0.8628***
Glutamate = 0.15 - 0.0001 IMI + 0.0003 EBR	- 0.3705	0.4769	0.6039*
Asparagine = 1.00 - 0.0009 IMI + 0.0041 EBR	- 0.2809	0.6272	0.6872*
Serine = 0.41 - 0.0004 IMI + 0.0003 EBR	- 0.6978	0.2992	0.7592**
Glutamine = 1.08 - 0.0018 IMI + 0.0017 EBR	- 0.8272	0.3911	0.9149***
Histidine = 2.18 - 0.0038 IMI + 0.0056 EBR	- 0.6698	0.4895	0.8296**
Glycine = 0.38 - 0.0002 IMI + 0.0003 EBR	- 0.2857	0.2162	0.3583
Arginine = 1.43 - 0.0029 IMI + 0.0033 EBR	- 0.7564	0.4244	0.8674***
Threonine = 0.11 - 8.33×10^{-5} IMI + 7×10^{-5} EBR	- 0.4083	0.1806	0.4465
Alanine = 0.52 - 0.0007 IMI + 0.0002 EBR	- 0.6931	0.1162	0.7027*
GABA = 0.02 - 3.69×10^{-7} IMI - 3×10^{-5}	- 0.0086	- 0.3966	0.3966
Tyrosine = 0.26 - 0.0001 IMI + 0.0002 EBR	- 0.3696	0.2343	0.4377
Cysteine = 0.14 - 0.0001 IMI + 0.0003 EBR	- 0.3918	0.4134	0.5697
Valine = 0.06 - 4.03×10^{-5} IMI + 9×10^{-6} EBR	- 0.4075	0.0446	0.4099
Methionine = 0.11 - 9.9×10^{-5} IMI + 0.0001 EBR	- 0.5309	0.3384	0.6296*
Tryptophan = 0.16 - 0.0002 IMI + 0.0003 EBR	- 0.4842	0.4119	0.6357*
Phenylalanine = 0.15 - 8.06×10^{-5} IMI + 6×10^{-5} EBR	- 0.3093	0.1152	0.3301
Isoleucine = 0.13 - 0.0002 IMI + 0.0003 EBR	- 0.5643	0.5121	0.7620**
Leucine = 0.11 - 2.05×10^{-5} IMI + 9×10^{-5} EBR	- 0.1068	0.2349	0.2580
Lysine = 0.13 - 0.0001 IMI + 0.0001 EBR	- 0.4396	0.2376	0.4997
Proline = 0.27 + 0.0002 IMI + 0.0008 EBR	0.3528	0.5735	0.6734*
TOTAL = 8.99 - 0.0119 IMI + 0.0184 EBR	**- 0.7136**	**0.5516**	**0.9020*** **

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively.

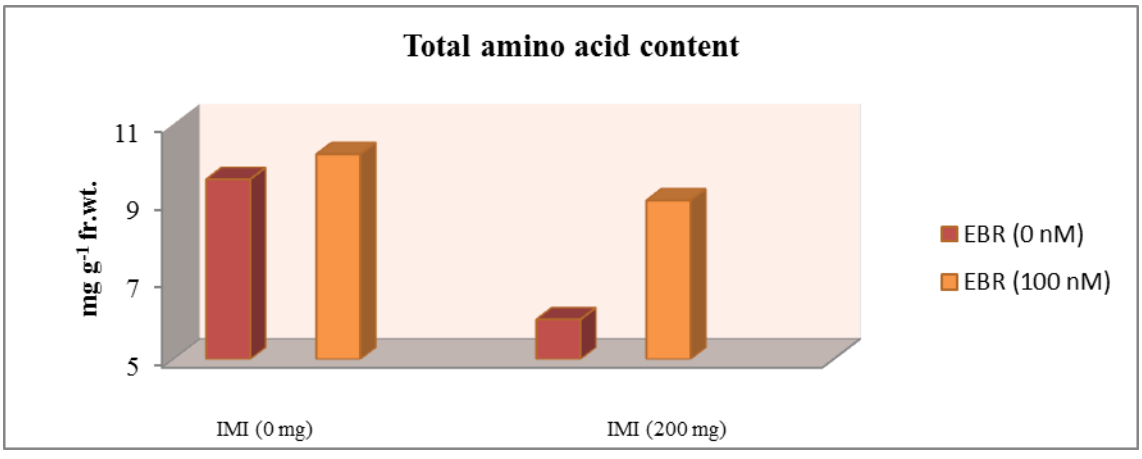

Fig. 7.2.1 Effect of seed soaking with EBR on total amino acid content in *B. juncea* seedlings grown under IMI toxicity.

Total amino acid content in the leaves of 30 days old control plants was 10.39 mg g^{-1} fr. wt. which was reduced to 7.86 mg g^{-1} fr. wt. in plants grown in IMI amended soils. Seed soaking with EBR before sowing and growing in IMI mixed soils resulted in increase of total amino acid content to 9.51 mg g^{-1} fr. wt. (Table 7.2.2, Fig. 7.2.2).

Table 7.2.2 Effect of seed soaking with EBR on amino acid content in the leaves of 30 day old *B. juncea* L. plants grown in IMI amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Name	**Treatments**				**F-ratios and HSD**			
IMI (mg Kg^{-1})	**0**	**0**	**300**	**300**				
EBR (nM L^{-1})	**0**	**100**	**0**	**100**				
Amino acid content (mg g^{-1} fr. wt.)					**F_{IMI}**	**F_{EBR}**	**$F_{IMI\times EBR}$**	**HSD**
Aspartate	0.155±0.035	0.160±0.032	0.130±0.012	0.141±0.025	2.07	0.25	0.03	0.07
Glutamate	0.166±0.032	0.171±0.016	0.165±0.027	0.185±0.028	0.19	0.61	0.24	0.06
Asparagine	1.194±0.035	1.215±0.147	0.910±0.145	1.046±0.123	10.45*	1.23	0.67	0.31
Serine	0.434±0.126	0.507±0.063	0.344±0.040	0.382±0.018	6.34*	1.68	0.17	0.19
Glutamine	1.116±0.316	1.238±0.095	0.794±0.158	1.076±0.065	5.07	3.54	0.56	0.48
Histidine	2.623±0.410	2.632±0.369	1.859±0.106	2.221±0.202	11.63**	1.15	1.04	0.78
Glycine	0.434±0.067	0.464±0.025	0.253±0.044	0.322±0.063	28.52***	2.73	0.42	0.14
Arginine	1.711±0.065	1.750±0.102	1.187±0.041	1.415±0.056	114.0***	11.01*	5.47*	0.18
Threonine	0.122±0.040	0.123±0.024	0.116±0.029	0.164±0.018	1.10	2.20	2.08	0.07
Alanine	0.679±0.079	0.648±0.026	0.441±0.127	0.572±0.084	9.87*	0.97	2.61	0.22
GABA	0.026±0.004	0.029±0.002	0.311±0.076	0.318±0.051	117.0***	0.03	0.008	0.12
Tyrosine	0.265±0.035	0.272±0.016	0.173±0.017	0.252±0.016	19.08**	11.06*	7.80*	0.06
Cysteine	0.194±0.040	0.208±0.040	0.139±0.023	0.150±0.015	9.82*	0.47	0.01	0.08
Valine	0.062±0.007	0.070±0.008	0.057±0.008	0.085±0.016	0.68	8.16*	2.89	0.03
Methionine	0.137±0.032	0.138±0.016	0.089±0.012	0.124±0.022	5.98*	2.09	1.85	0.05
Tryptophan	0.155±0.018	0.162±0.029	0.104±0.029	0.116±0.021	11.58**	0.42	0.035	0.06
Phenylalanine	0.181±0.013	0.185±0.035	0.121±0.007	0.128±0.017	23.81**	0.17	0.02	0.05
Isoleucine	0.156±0.035	0.199±0.046	0.056±0.012	0.059±0.015	47.47***	1.68	1.36	0.08
Leucine	0.143±0.031	0.157±0.018	0.139±0.035	0.145±0.019	0.28	0.41	0.08	0.07
Lysine	0.154±0.025	0.154±0.041	0.152±0.016	0.153±0.031	0.006	0.0003	0.0003	0.07
Proline	0.285±0.043	0.304±0.084	0.320±0.036	0.466±0.077	7.23*	4.99	2.97	0.16
TOTAL	**10.394±0.184**	**10.784±0.379**	**7.861±0.211**	**9.517±0.177**	**171.2*** **	**49.45*** **	**18.97** **	**0.65**

Multiple linear regression			
MLR equation	**β-regression coefficients**		**Multiple correlation coefficient**
	β_{IMI}	**β_{EBR}**	
Aspartate = 0.15 - 0.0001 IMI + 8×10^{-5} EBR	- 0.4471	0.1565	0.4737
Glutamate = 0.16 + 3.38×10^{-5} IMI + 0.0001 EBR	0.1473	0.2610	0.2997
Asparagine = 1.16 - 0.0011 IMI + 0.0008 EBR	- 0.7163	0.2465	0.7576**
Serine = 0.44 - 0.0005 IMI + 0.0006 EBR	- 0.6256	0.3224	0.7038*
Glutamine = 1.07 - 0.0012 IMI + 0.002 EBR	- 0.5431	0.4544	0.7082*
Histidine = 2.53 - 0.0029 IMI + 0.0019 EBR	- 0.7299	0.2300	0.7653**
Glycine = 0.42 - 0.0008 IMI + 0.0005 EBR	- 0.8478	0.2626	0.8875***
Arginine = 1.66 - 0.0021 IMI + 0.0013 EBR	- 0.9073	0.2818	0.9501***
Threonine = 0.11 + 8.7×10^{-5} IMI + 0.0002 EBR	0.2866	0.4056	0.4966
Alanine = 0.62 - 0.0007 IMI + 0.0005 EBR	- 0.6781	0.2135	0.7110*
GABA = 0.02 + 0.0014 IMI + 5×10^{-5} EBR	0.9673	0.0165	0.9674***
Tyrosine = 0.2 - 0.0002 IMI + 0.0004 EBR	- 0.6444	0.4906	0.8099**
Cysteine = 0.19 - 0.0002 IMI + 0.0001 EBR	- 0.7324	0.1617	0.7500**
Valine = 0.05 + 2.51×10^{-5} IMI + 0.0002 EBR	0.1857	0.6430	0.6693*
Methionine = 0.12 - 0.0001 IMI + 0.0002 EBR	- 0.5776	0.3414	0.6710*
Tryptophan = 0.153 - 0.0002 IMI + 9×10^{-5} EBR	- 0.7602	0.1458	0.7741**
Phenylalanine = 0.18 - 0.0003 IMI + 5×10^{-5} EBR	- 0.8626	0.0729	0.8657***
Isoleucine = 0.16 - 0.0006 IMI + 0.0002 EBR	- 0.9006	0.1697	0.9165***
Leucine = 0.14 - 4.13×10^{-5} IMI + 1×10^{-4} EBR	- 0.1811	0.2166	0.2824
Lysine = 0.15 - 7.07×10^{-6} IMI + 3×10^{-6} EBR	- 0.0289	0.0065	0.0297
Proline = 0.25 + 0.0005 IMI + 0.0008 EBR	0.5583	0.4638	0.7258*
TOTAL = 10.07 - 0.0095 IMI + 0.0102 EBR	**- 0.8311**	**0.4475**	**0.9440*** **

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively.

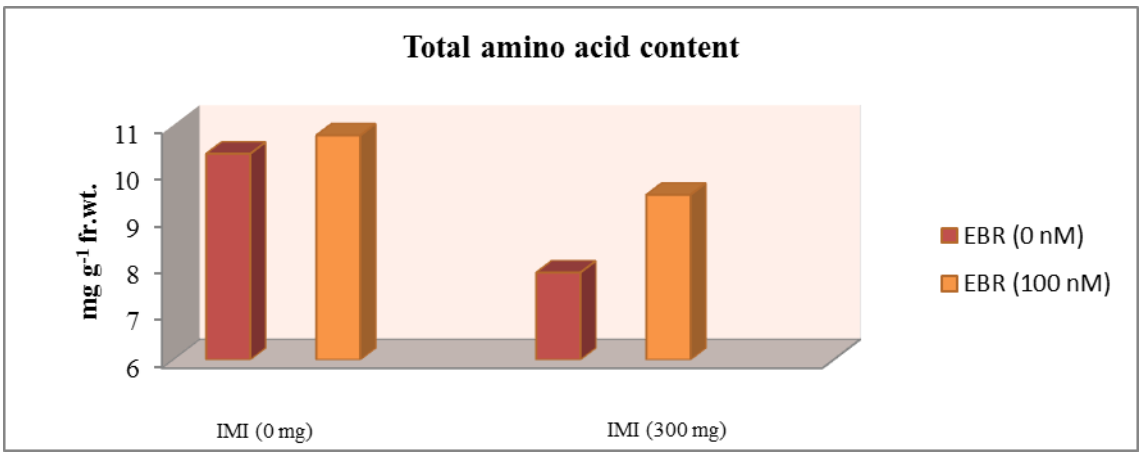

Fig. 7.2.2 Effect of seed soaking with EBR on total amino acid content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In 60 days old *B. juncea* plants, total amino acid content was maximum reduced to 8.77 mg g^{-1} fr. wt. in the leaves of plants grown in soils amended with IMI, when compared to control plants (12.07 mg g^{-1} fr. wt.). But recovery in total amino acid content (10.89g g^{-1} fr. wt.) was observed in plants raised from EBR treated seeds and grown in pots containing IMI (Table 7.2.3, Fig. 7.2.3).

Table 7.2.3 Effect of seed soaking with EBR on amino acid content in the leaves of 60 day old *B. juncea* L. plants grown in IMI amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Name	**Treatments**				**F-ratios and HSD**			
IMI (mg Kg^{-1})	**0**	**0**	**300**	**300**				
EBR (nM L^{-1})	**0**	**100**	**0**	**100**				
Amino acid content (mg g^{-1} fr. wt.)								
Aspartate	0.249±0.039	0.250±0.040	0.242±0.062	0.245±0.070	0.04	0.004	0.0006	0.14
Glutamate	0.190±0.032	0.192±0.047	0.163±0.032	0.244±0.027	0.37	4.16	3.74	0.09
Asparagine	1.333±0.185	1.367±0.129	0.985±0.256	1.327±0.190	2.94	2.77	1.85	0.51
Serine	0.554±0.059	0.581±0.101	0.336±0.056	0.467±0.062	15.91**	3.63	1.55	0.18
Glutamine	1.271±0.084	1.394±0.210	0.993±0.121	1.070±0.048	15.84**	1.75	0.09	0.34
Histidine	3.394±0.197	3.380±0.351	1.637±0.139	2.094±0.100	144.85***	3.05	3.47	0.57
Glycine	0.450±0.043	0.455±0.023	0.391±0.094	0.601±0.023	1.94	11.72**	10.72*	0.14
Arginine	1.719±0.065	1.785±0.129	1.297±0.083	1.678±0.296	7.25*	5.17	2.57	0.44
Threonine	0.150±0.034	0.161±0.049	0.137±0.014	0.155±0.031	0.23	0.54	0.03	0.09
Alanine	0.803±0.153	0.845±0.120	0.839±0.024	1.014±0.117	2.43	2.70	1.01	0.30
GABA	0.027±0.008	0.033±0.004	0.030±0.005	0.048±0.004	8.75*	14.67**	3.41	0.01
Tyrosine	0.371±0.033	0.391±0.068	0.351±0.044	0.377±0.045	0.35	0.64	0.01	0.13
Cysteine	0.194±0.032	0.196±0.024	0.109±0.008	0.118±0.017	41.07***	0.21	0.08	0.05
Valine	0.071±0.003	0.075±0.004	0.037±0.007	0.039±0.007	119.20***	0.72	0.08	0.02
Methionine	0.173±0.052	0.170±0.039	0.155±0.027	0.160±0.023	0.44	0.002	0.03	0.09
Tryptophan	0.162±0.037	0.175±0.048	0.136±0.012	0.139±0.018	2.70	0.20	0.07	0.08
Phenylalanine	0.186±0.020	0.191±0.065	0.106±0.025	0.123±0.021	11.71**	0.25	0.07	0.09
Isoleucine	0.162±0.018	0.169±0.038	0.131±0.031	0.154±0.025	1.82	0.83	0.22	0.07
Leucine	0.146±0.047	0.167±0.015	0.159±0.028	0.212±0.021	2.74	4.35	0.84	0.08
Lysine	0.160±0.034	0.161±0.039	0.146±0.007	0.166±0.035	0.04	0.31	0.25	0.08
Proline	0.312±0.058	0.316±0.010	0.396±0.072	0.467±0.020	18.12**	1.84	1.50	0.12
TOTAL	**12.076±0.073**	**12.452±0.472**	**8.778±0.412**	**10.899±0.527**	**104.61*** **	**27.71*** **	**13.52****	**1.07**

Multiple linear regression			
MLR equation	**β-regression coefficients**		**Multiple correlation coefficient**
	β_{IMI}	**β_{EBR}**	
Aspartate = 0.24 - 3.34×10^{-5} IMI + 2×10^{-5} EBR	- 0.0750	0.0223	0.0782
Glutamate = 0.17 + 6.26×10^{-5} IMI + 0.0004 EBR	0.1517	0.5058	0.5280
Asparagine = 1.25 - 0.0009 IMI + 0.0019 EBR	- 0.4346	0.4222	0.6059*
Serine = 0.52 - 0.0008 IMI + 0.0008 EBR	- 0.7394	0.3534	0.8195**
Glutamine = 1.28 - 0.0015 IMI + 0.001 EBR	- 0.7852	0.2615	0.8276**
Histidine = 3.27 - 0.0076 IMI + 0.0022 EBR	- 0.9533	0.1385	0.9633***
Glycine = 0.39 + 0.0002 IMI + 0.0011 EBR	0.2451	0.6016	0.6496*
Arginine = 1.64 - 0.0013 IMI + 0.0022 EBR	- 0.5616	0.4741	0.7350**
Threonine = 0.14 - 4.76×10^{-5} IMI + 0.0001 EBR	- 0.1626	0.2482	0.2967
Alanine = 0.76 + 0.0005 IMI + 0.0011 EBR	0.4145	0.4372	0.6025*
GABA = 0.02 + 4.63×10^{-5} IMI + 0.0001 EBR	0.5012	0.6489	0.8200**
Tyrosine = 0.36 - 8.44×10^{-5} IMI + 0.0002 EBR	- 0.1978	0.2684	0.3335
Cysteine = 0.19 - 0.0004 IMI + 6×10^{-5} EBR	- 0.9121	0.0654	0.9144***
Valine = 0.07 - 0.0002 IMI + 3×10^{-5} EBR	- 0.9649	0.0753	0.9679***
Methionine = 0.17 - 7.08×10^{-5} IMI + 9×10^{-6} EBR	- 0.2280	0.0143	0.2284
Tryptophan = 0.16 - 0.0001 IMI + 8×10^{-5} EBR	- 0.4965	0.1361	0.5148
Phenylalanine = 0.18 - 0.0004 IMI + 0.0001 EBR	- 0.7644	0.1135	0.7728**
Isoleucine = 0.15 - 0.0001 IMI + 0.0002 EBR	- 0.4098	0.2767	0.4945
Leucine = 0.13 + 0.0001 IMI + 0.0004 EBR	0.4149	0.5227	0.6673*
Lysine = 0.15 - 2.02×10^{-5} IMI + 0.0001 EBR	- 0.0753	0.1898	0.2042
Proline = 0.29 + 0.0006 IMI + 0.0004 EBR	0.7841	0.2502	0.8231**
TOTAL = 11.64 - 0.0121 IMI + 0.0125 EBR	**- 0.8246**	**0.4244**	**0.9274*** **
*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively.			

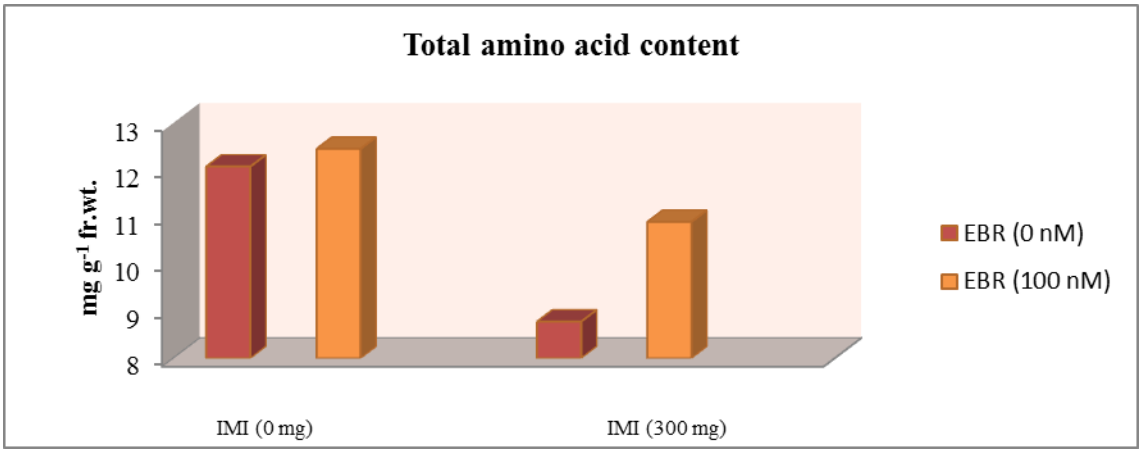

Fig. 7.2.3 Effect of seed soaking with EBR on total amino acid content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In the leaves of 90 days old plants of *B. juncea* grown under IMI toxicity, the total amino acid content was reduced to 4.43 mg g^{-1} fr. wt. when compared to 4.95 mg g^{-1} fr. wt. in control plants. Seed soaking with EBR before sowing resulted in increase in total amino acid content to 4.80 mg g^{-1} fr. wt. in the leaves of plants grown in soils amended with IMI (Table 7.2.4, Fig. 7.2.4).

Table 7.2.4 Effect of seed soaking with EBR on amino acid content in the leaves of 90 day old *B. juncea* L. plants grown in IMI amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Name	Treatments				F-ratios and HSD			
IMI (mg Kg^{-1})	0	0	300	300				
EBR (nM L^{-1})	0	100	0	100				
Amino acid content (mg g^{-1} fr. wt.)								
Aspartate	0.094±0.010	0.097±0.018	0.085±0.016	0.093±0.013	0.65	0.39	0.07	0.04
Glutamate	0.072±0.015	0.078±0.010	0.068±0.009	0.071±0.016	0.47	0.35	0.04	0.03
Asparagine	0.389±0.051	0.392±0.068	0.333±0.077	0.377±0.078	0.80	0.33	0.25	0.18
Serine	0.238±0.053	0.240±0.054	0.191±0.021	0.217±0.068	1.36	0.21	0.15	0.13
Glutamine	0.424±0.040	0.487±0.077	0.402±0.063	0.402±0.047	2.49	0.88	0.87	0.15
Histidine	1.251±0.061	1.260±0.056	1.167±0.040	1.205±0.083	3.72	0.42	0.15	0.16
Glycine	0.195±0.028	0.195±0.038	0.169±0.042	0.178±0.029	1.10	0.05	0.04	0.09
Arginine	0.888±0.081	0.943±0.059	0.746±0.138	0.868±0.181	2.28	1.54	0.21	0.32
Threonine	0.090±0.013	0.107±0.039	0.081±0.016	0.082±0.016	1.52	0.42	0.34	0.06
Alanine	0.274±0.024	0.387±0.083	0.215±0.079	0.263±0.079	5.00	3.89	0.62	0.18
GABA	0.011±0.001	0.011±0.006	0.009±0.001	0.010±0.002	0.47	0.08	0.001	0.01
Tyrosine	0.065±0.005	0.074±0.006	0.046±0.005	0.053±0.014	17.04**	2.60	0.03	0.02
Cysteine	0.103±0.037	0.119±0.031	0.100±0.031	0.098±0.029	0.38	0.15	0.23	0.08
Valine	0.027±0.002	0.037±0.008	0.035±0.008	0.035±0.004	0.37	1.94	2.05	0.02
Methionine	0.082±0.011	0.087±0.002	0.081±0.017	0.080±0.023	0.16	0.05	0.15	0.04
Tryptophan	0.076±0.007	0.078±0.005	0.060±0.018	0.065±0.021	2.82	0.16	0.03	0.04
Phenylalanine	0.099±0.029	0.104±0.020	0.099±0.021	0.107±0.022	0.007	0.21	0.007	0.06
Isoleucine	0.120±0.030	0.120±0.013	0.090±0.022	0.091±0.021	5.21	0.01	0.007	0.06
Leucine	0.115±0.008	0.114±0.018	0.098±0.025	0.112±0.019	0.76	0.40	0.49	0.05
Lysine	0.118±0.016	0.121±0.008	0.104±0.021	0.116±0.038	0.46	0.29	0.12	0.06
Proline	0.226±0.043	0.238±0.045	0.251±0.033	0.280±0.028	2.24	0.85	0.14	0.1
TOTAL	**4.955±0.102**	**5.291±0.078**	**4.431±0.123**	**4.803±0.371**	**18.10****	**8.86***	**0.02**	**0.53**

Multiple linear regression and artificial neural network analysis

MLR equation	β-regression coefficients		Multiple correlation coefficient
	β_{IMI}	β_{EBR}	
Aspartate = $0.09 - 3.39\times10^{-5} + 5\times10^{-5}$ EBR	- 0.2680	0.2086	0.3396
Glutamate = $0.07 - 2.58\times10^{-5}$ IMI + 4×10^{-5} EBR	- 0.2308	0.1993	0.3049
Asparagine = 0.37 - 0.0002 IMI + 0.0002 EBR	- 0.2922	0.1897	0.3484
Serine = 0.23 - 0.0002 IMI + 0.0001 EBR	- 0.3747	0.1476	0.4028
Glutamine = 0.43 - 0.0003 IMI + 0.0003 EBR	- 0.4515	0.2679	0.5250
Histidine = 1.24 - 0.0003 IMI + 0.0002 EBR	- 0.5502	0.1857	0.5807
Glycine = 0.19 - 0.0001 IMI + 5×10^{-5} EBR	- 0.3462	0.0799	0.3553
Arginine = 0.87 - 0.0005 IMI + 0.0009 EBR	- 0.4355	0.3581	0.5638
Threonine = 0.09 - 8.39×10^{-5} IMI + 9×10^{-5} EBR	- 0.3850	0.2042	0.4357
Alanine = 0.29 - 0.0005 IMI + 0.0008 EBR	- 0.5342	0.4716	0.7126*
GABA = 0.01 - 6.95×10^{-6} IMI + 6×10^{-6} EBR	- 0.2353	0.1015	0.2563
Tyrosine = 0.06 - 9.93×10^{-5} IMI + 8×10^{-5} EBR	- 0.7848	0.3065	0.8425**
Cysteine = 0.10 - 5.74×10^{-5} IMI + 7×10^{-5} EBR	- 0.2095	0.1347	0.2491
Valine = 0.03 + 1.08×10^{-5} IMI + 5×10^{-5} EBR	0.1741	0.3963	0.4328
Methionine = 0.08 - 1.82×10^{-5} IMI + 2×10^{-5} EBR	- 0.1409	0.0784	0.1613
Tryptophan = 0.07 - 6.98×10^{-5} IMI + 3×10^{-5} EBR	- 0.5063	0.1225	0.5209
Phenylalanine = 0.09 + 5.9×10^{-6} IMI + 6×10^{-5} EBR	0.0307	0.1630	0.1659
Isoleucine = 0.11 - 0.0001 IMI + 1×10^{-5} EBR	- 0.6277	0.0277	0.6283*
Leucine = 0.11 - 4.63×10^{-5} IMI + 7×10^{-5} EBR	- 0.2812	0.2044	0.3776
Lysine = 0.11 - 4.63×10^{-5} IMI + 7×10^{-5} EBR	- 0.2295	0.1814	0.2926
Proline = 0.22 + 0.0002 IMI + 0.0002 EBR	0.4462	0.2761	0.5248
TOTAL = 4.94 - 0.0025 IMI + 0035 EBR	**- 0.7192**	**0.5033**	**0.8778*****

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively.

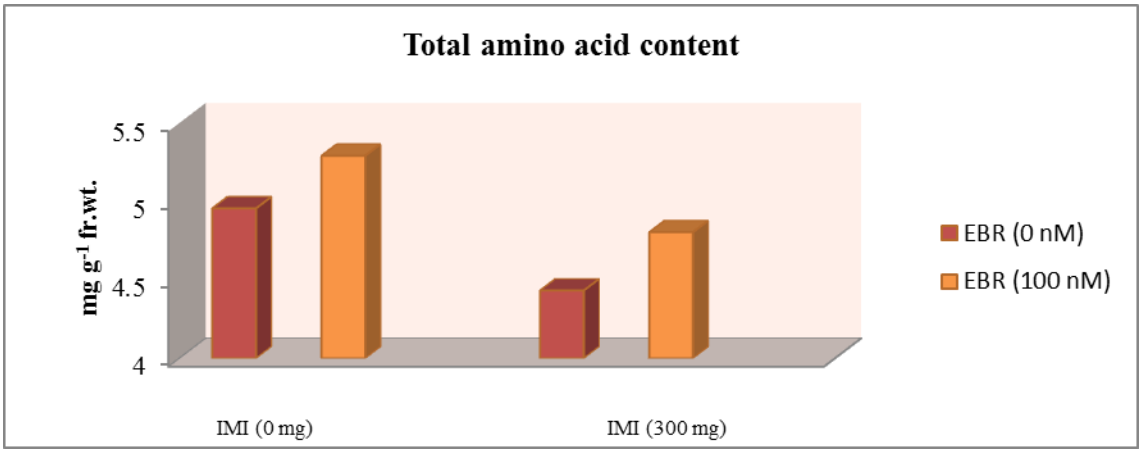

Fig. 7.2.4 Effect of seed soaking with EBR on total amino acid content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Two-way ANOVA and Tukey's HSD showed significant differences for total amino acid contents in the seedlings and leaves of *B. juncea* plants raised from EBR treated seeds and grown under IMI toxicity. MLR analysis also revealed that IMI application reduced the total amino acid contents (indicated by negative β-regression coefficients), whereas EBR seed soaking enhanced the total amino acid contents (indicated by positive β-regression coefficients) as shown in table 7.2.1, 7.2.2, 7.2.3 and 7.2.4. High correlation between simulated and experimental data was observed after analyzing the data using ANN model (Fig. 7.2.5)

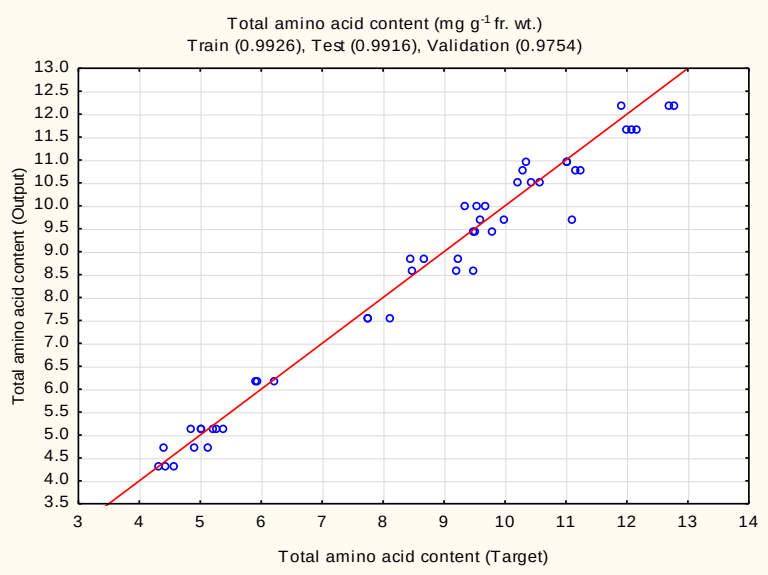

Fig. 7.2.5 Correlation between target (experimental) and output (simulated) total amino acid contents using ANN model (p<0.001).

7.3 Organic acid analysis

Citrate

Content of citrate in 10 days old *B. juncea* seedlings (Control) was 3.21 mg g^{-1} fr. wt. which was maximum enhanced to 5.26 mg g^{-1} fr. wt. in the seedlings raised from seeds soaked with 100 nM EBR followed by growing in Petri-plates supplemented with 200 mg IMI L^{-1} (Table 7.3.1, Fig. 7.3.1).

Table 7.3.1 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on citrate content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates (Mean ± SD, Two-way ANOVA, Tukey's HSD, multiple linear regression (MLR).

Treatments		Citrate content (mg g^{-1} dr. wt.)		
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	3.21±0.45		
0	100	3.86±0.12		
150	0	3.81±0.31		
150	100	4.32±0.31		
200	0	4.37±0.21		
200	100	5.26±0.51		
250	0	3.40±0.08		
250	100	3.80±0.29		
Two-way ANOVA				
F_{IMI}		20.8***		
F_{EBR}		22.5***		
$F_{IMI \times EBR}$		0.67		
HSD		0.85		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Citrate = 3.39 + 0.0020 IMI + 0.0061 EBR		0.2880	0.4676	0.5492**
** and *** indicate significant at p<0.01 and p<0.001 respectively. r = multiple correlation coefficient.				

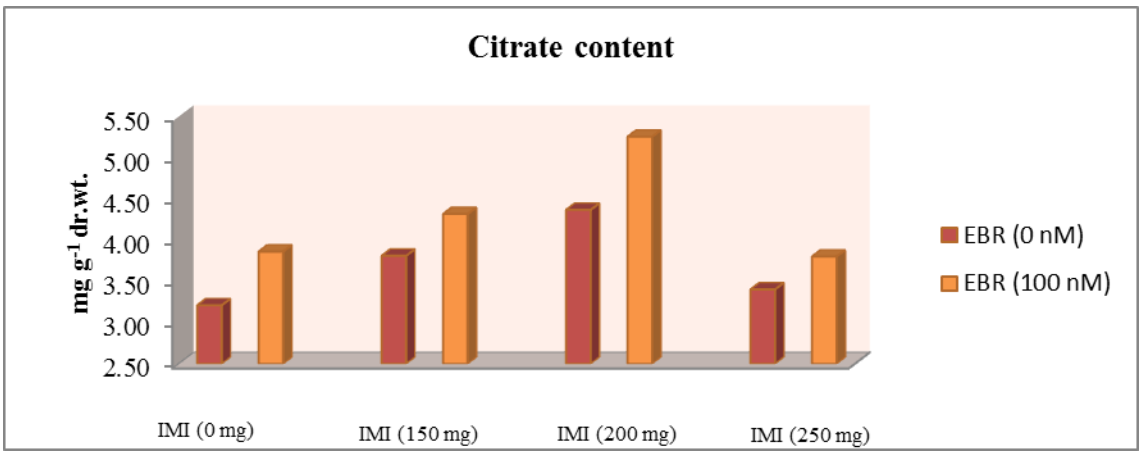

Fig. 7.3.1 Effect of seed soaking with EBR on citrate content in *B. juncea* seedlings grown under IMI toxicity.

In the leaves of 30 days old plants, as compared to control (21.91 mg g^{-1} fr. wt.), citrate content was maximum enhanced to 64.46 mg g^{-1} fr. wt. in the plants raised from EBR (100 nM) treated seeds and grown in pots containing 300 mg IMI Kg^{-1} soil (Table 7.3.2, Fig. 7.3.2).

Table 7.3.2 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on citrate content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Citrate content (mg g^{-1} dr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	21.91±2.95	39.70±2.74	45.48±2.10
0	100	33.90±0.44	66.26±11.99	41.08±5.40
250	0	23.30±1.61	51.94±12.38	66.10±21.38
250	100	51.13±8.61	57.10±6.32	66.48±12.80
300	0	53.51±1.72	62.48±2.90	71.18±11.62
300	100	64.46±15.28	68.72±9.49	71.51±16.62
350	0	27.81±3.11	54.97±2.86	52.16±8.89
350	100	57.21±0.66	84.85±2.04	76.95±22.07
Two-way ANOVA				
F_{IMI}		24.5***	7.2**	4.52*
F_{EBR}		57.9***	30.2***	0.81
$F_{IMI \times EBR}$		3.5*	4.5*	1.28
HSD		18.2	21.3	40.40
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Citrate (30 DAS) = 18.01 + 0.0605 IMI + 0.2004 EBR		0.4923	0.6052	0.7801***
Citrate (60 DAS) = 42.65 + 0.0427 IMI + 0.1696 EBR		0.4138	0.6093	0.7366***
Citrate (90 DAS) = 42.16 + 0.0736 IMI + 0.0527 EBR		0.5813	0.1546	0.6015**

*, ** and *** indicate significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. r = multiple correlation coefficient. DAS = days after sowing.

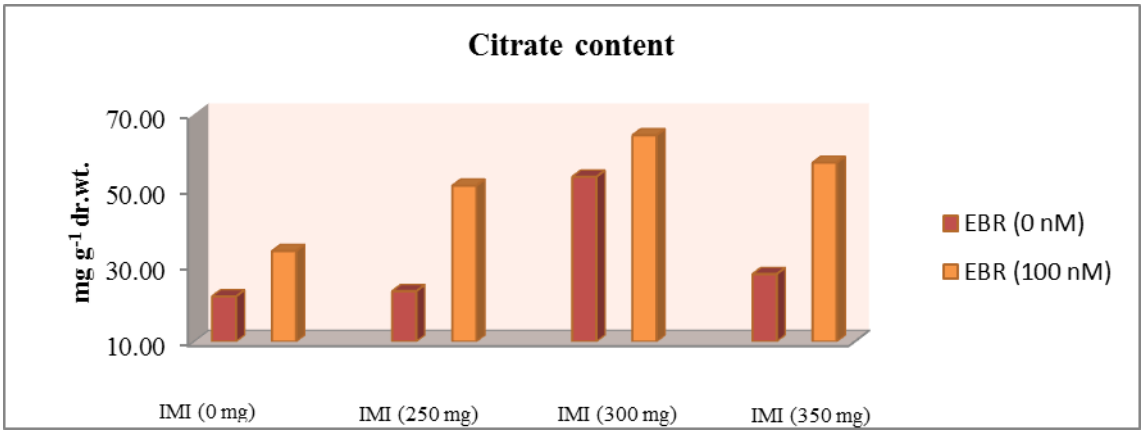

Fig. 7.3.2 Effect of seed soaking with EBR on citrate content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Citrate content observed in the leaves of 60 days old control plants was 39.70 mg g^{-1} fr. wt. which got enhanced to 84.85 mg g^{-1} fr. wt. in plants raised from 100 nM EBR soaked seeds and grown under IMI toxicity (350 mg IMI Kg^{-1} soil) (Table 7.3.2, Fig. 7.3.3).

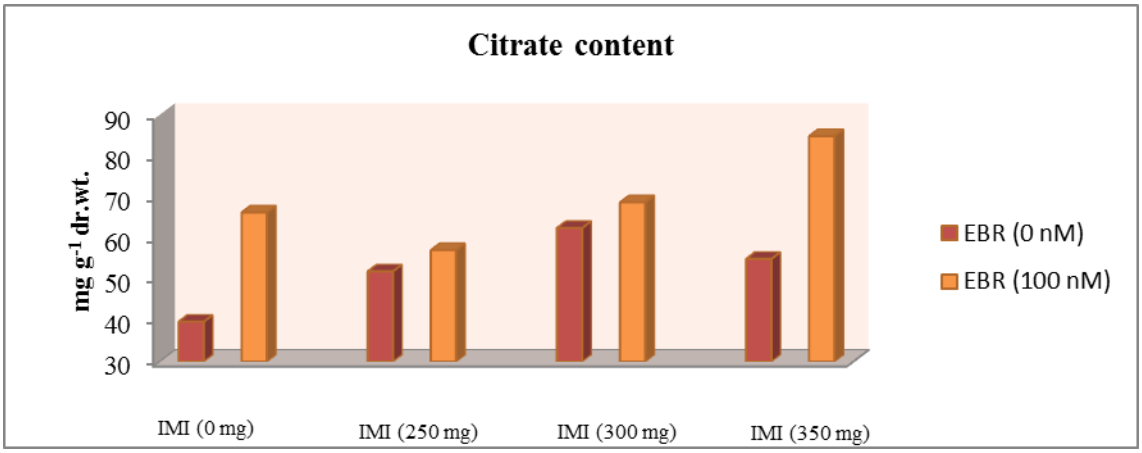

Fig. 7.3.3 Effect of seed soaking with EBR on citrate content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In the mature leaves of *B. juncea* plants (90 DAS), maximum citrate content observed was 76.95 mg g^{-1} fr. wt. in plants germinated from EBR (100 nM) treated seeds and grown in pots containing 350 mg IMI Kg^{-1} soil, when compared with citrate content (45.48 mg g^{-1} fr. wt.) in control plants (Table 7.3.2, Fig. 7.3.4).

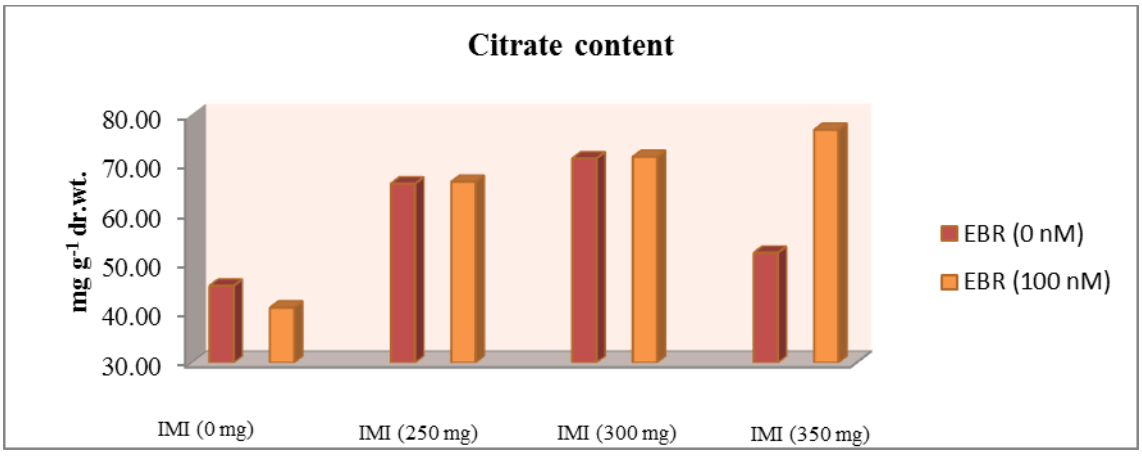

Fig. 7.3.4 Effect of seed soaking with EBR on citrate content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Data analysis using two-way ANOVA and Tukey's HSD showed that contents of citrate were significantly different in seedlings and leaves of *B. juncea* plants. MLR analysis revealed that both of IMI as well as EBR application resulted in increase of citrate contents in *B. juncea* as indicated by positive β-regression coefficients (Tables 7.3.1 and 7.3.2). ANN model also revealed that simulated (output) and experimental (target) data is highly correlated (Fig. 7.3.5).

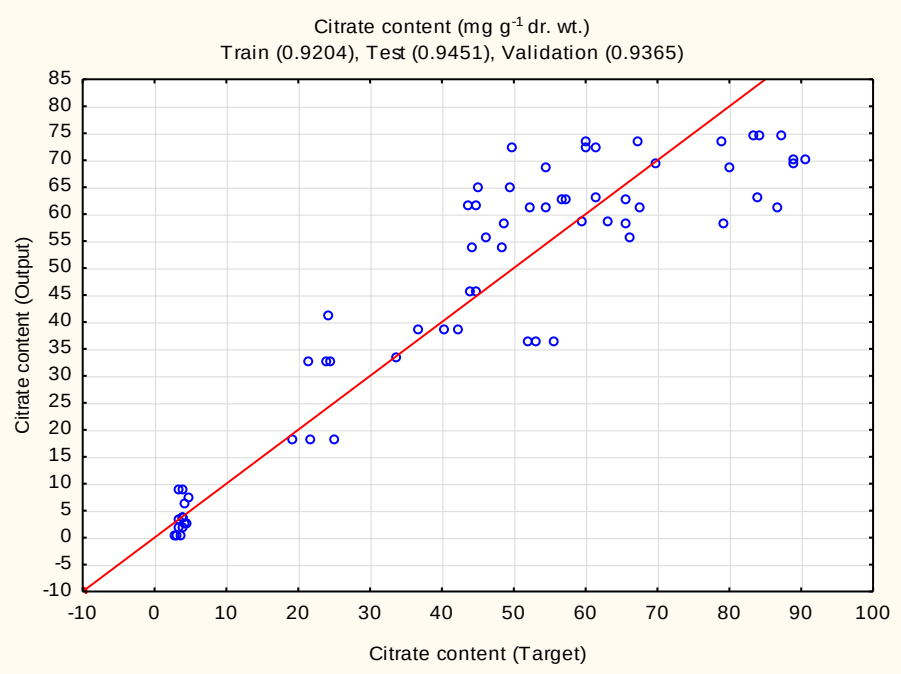

Fig. 7.3.5 Correlation between target (experimental) and output (simulated) citrate contents using ANN model ($p<0.001$).

Succinate

In 10 days old seedlings (Control), succinate content observed was 0.85 mg g^{-1} dr. wt. which was maximum increased to 1.08 mg g^{-1} dr. wt. in seedlings germinated from EBR (100 nM) soaked seeds and grown in Petri-plates containing 200 mg IMI L^{-1} solutions (Table 7.3.3, Fig. 7.3.6).

Table 7.3.3 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on succinate content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Succinate content (mg g^{-1} dr. wt.)		
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	0.85±0.01		
0	100	0.96±0.04		
150	0	0.94±0.05		
150	100	1.00±0.04		
200	0	0.97±0.02		
200	100	1.08±0.04		
250	0	0.86±0.03		
250	100	0.88±0.02		
Two-way ANOVA				
F_{IMI}		24.7***		
F_{EBR}		27.8***		
$F_{IMI \times EBR}$		2.16		
HSD		0.089		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	**β_{EBR}**	
Succinate = 0.89 + 7.15×10^{-5} IMI + 0.0007 EBR		0.0864	0.4706	0.4785*
* and *** indicate significant at p<0.05 and p<0.001 respectively. r = multiple correlation coefficient.				

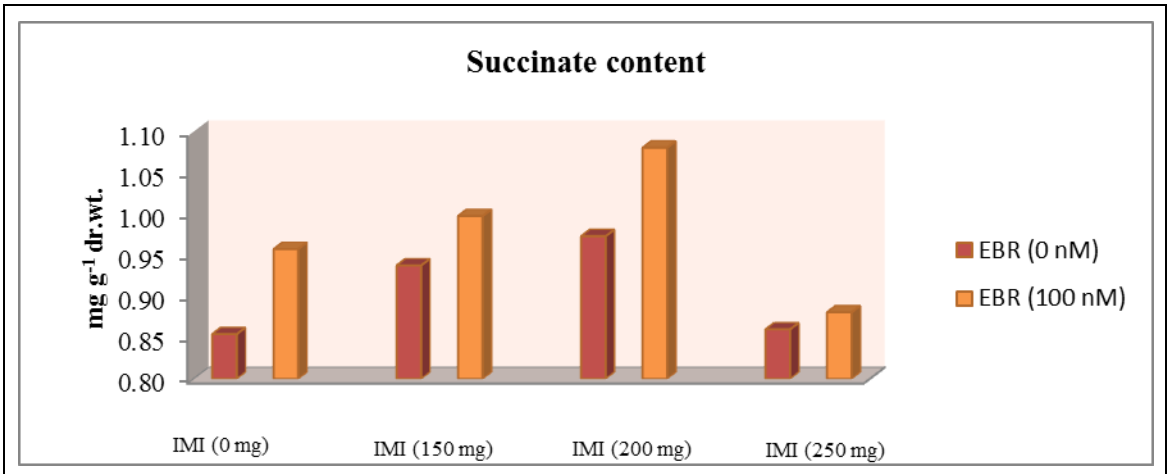

Fig. 7.3.6 Effect of seed soaking with EBR on succinate content in *B. juncea* seedlings grown under IMI toxicity.

Content of succinate in the leaves of 30 days old *B. juncea* control plants was 0.94 mg g^{-1} dr. wt. and it was enhanced to 1.29 mg g^{-1} dr. wt. in plants raised from 100 nM EBR treated seeds and grown in pots containing 300 mg IMI Kg^{-1} soil (Table 7.3.4, Fig. 7.3.7).

Table 7.3.4 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on succinate content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Succinate content (mg g^{-1} dr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	0.94±0.07	6.37±1.77	2.38±0.35
0	100	0.93±0.07	7.38±1.58	4.01±1.22
250	0	0.83±0.02	4.75±1.01	2.33±0.55
250	100	0.94±0.06	6.36±0.43	3.71±0.40
300	0	1.10±0.04	4.53±0.70	1.68±0.35
300	100	1.29 ± 0.33	6.46±0.71	2.40±0.20
350	0	0.97±0.13	7.19±2.94	3.23±0.36
350	100	1.17±0.04	10.62±0.44	3.90±0.97
Two-way ANOVA				
F_{IMI}		6.5**	7.3**	6.13**
F_{EBR}		5.2*	11.3**	17.53***
$F_{IMI \times EBR}$		0.7	0.75	0.83
HSD		0.36	4.08	1.82
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Succinate (30 DAS) = 0.84 + 0.0005 IMI + 0.0012 EBR		0.3682	0.3456	0.5050**
Succinate (60 DAS) = 5.34 + 0.0016 IMI + 0.0199 EBR		0.1024	0.4691	0.4802*
Succinate (90 DAS) = 2.54 - 0.0006 IMI + 0.011 EBR		- 0.0849	0.5678	0.5738**

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

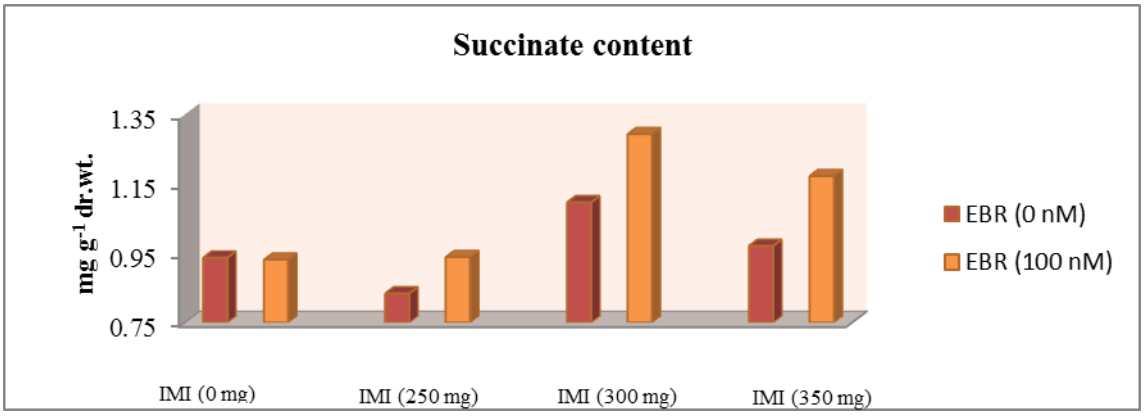

Fig. 7.3.7 Effect of seed soaking with EBR on succinate content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In the leaves of 60 days old plants raised from EBR (100 nM) treated seeds and grown in soils amended with 350 mg IMI Kg^{-1} soil, succinate content observed was 10.62 mg g^{-1} dr. wt. as compared to succinate content in control plants (6.37 mg g^{-1} dr. wt.) (Table 7.3.4, Fig. 7.3.8).

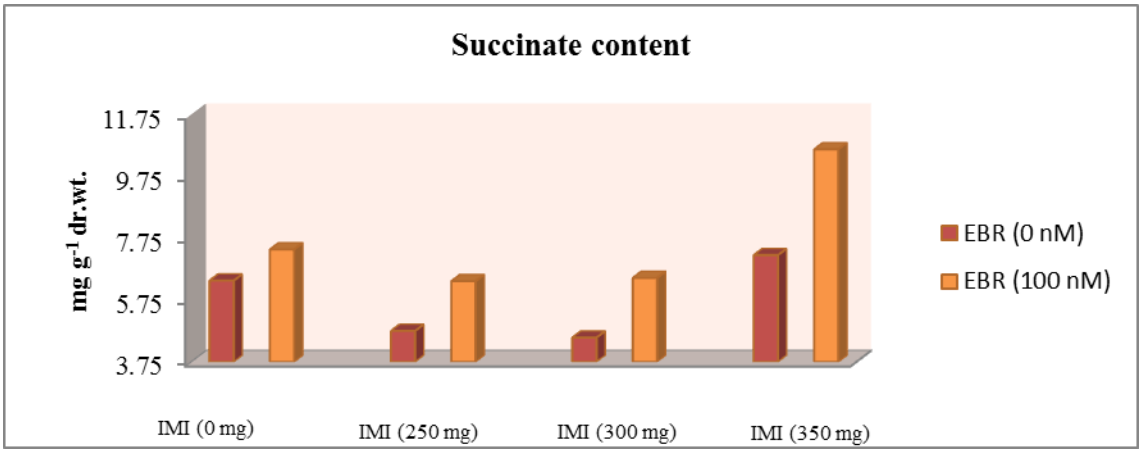

Fig. 7.3.8 Effect of seed soaking with EBR on succinate content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In the leaves of *B. juncea* (90 DAS), succinate content in control plants was 2.38 mg g^{-1} dr. wt. which was increased to 3.90 mg g^{-1} dr. wt. in the plants raised from EBR (100 nM) treated seeds and grown in pots containing 350 mg IMI Kg^{-1} soil (Table 7.3.4, Fig. 7.3.9).

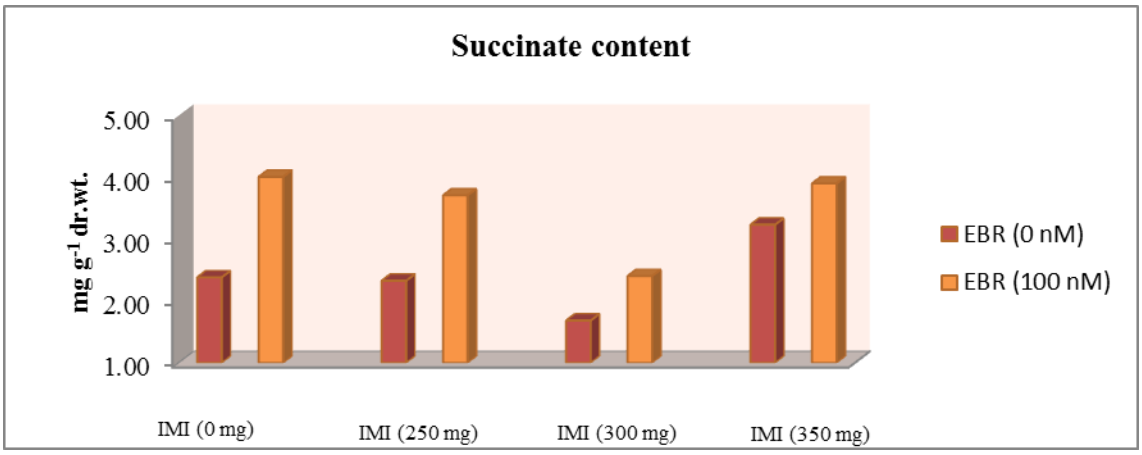

Fig. 7.3.9 Effect of seed soaking with EBR on succinate content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Statistical analysis using two-way ANOVA and Tukey's HSD showed that succinate contents were statistically different for control, EBR and IMI treated *B. juncea* plants. MLR analysis revealed that both of IMI application and EBR seeds soaking enhanced the contents of succinate in *B. juncea* as indicated by positive β-regression values (Tables 7.3.3 and 7.3.4). It was observed that ANN model simulated the experimental data at very high level of significance ($p<0.001$) (Fig. 7.3.10).

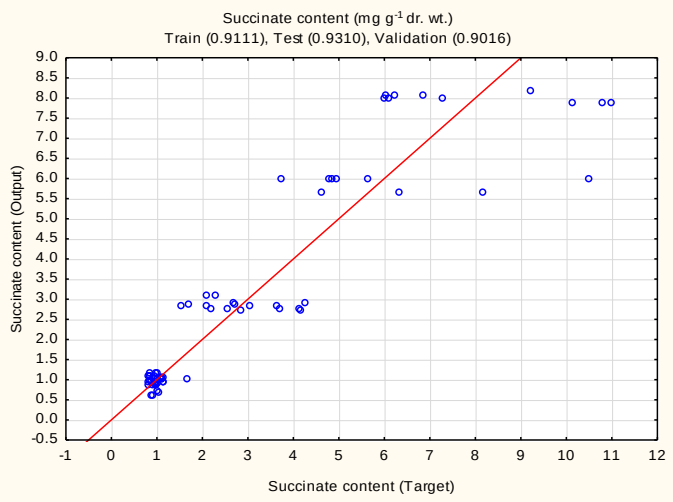

Fig. 7.3.10 Correlation between target (experimental) and output (simulated) succinate contents using ANN model (p<0.001).

Fumarate

In comparison to control seedlings (10 DAS), fumarate content observed was 0.3709 mg g^{-1} dr. wt.), which increased to 0.3832 mg g^{-1} dr. wt. in seedlings raised from 100 nM EBR treated seeds and grown in Petri-plates containing 200 mg IMI L^{-1} solutions (Table 7.3.5, Fig. 7.3.11).

Table 7.3.5 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on fumarate content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Fumarate content (mg g^{-1} dr. wt.)		
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	0.3709±0.0006		
0	100	0.3746±0.0003		
150	0	0.3727±0.0032		
150	100	0.3765±0.0030		
200	0	0.3757±0.0012		
200	100	0.3832±0.0019		
250	0	0.3782±0.0008		
250	100	0.3784±0.0002		
Two-way ANOVA				
F_{IMI}		18.3***		
F_{EBR}		27.3***		
$F_{IMI \times EBR}$		4.09*		
HSD		0.005		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Fumarate = 0.37 + 2.55×10^{-5} IMI + 4×10^{-5} EBR		0.6176	0.4971	0.7928***
* and *** indicate significant at p<0.05 and p<0.001 respectively. r = multiple correlation coefficient.				

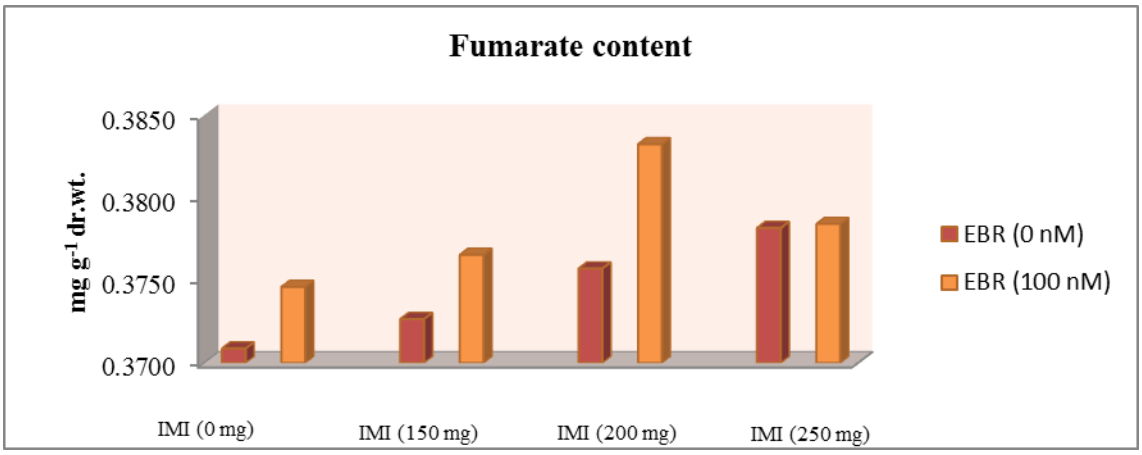

Fig. 7.3.11 Effect of seed soaking with EBR on fumarate content in *B. juncea* seedlings grown under IMI toxicity.

Fumarate in the leaves of 30 days old control plants was 0.38 mg g^{-1} dr. wt., which was maximum enhanced to 0.51 mg g^{-1} dr. wt. in the leaves of plants raised from 100 nM EBR soaked seeds sown in soils amended with 300 mg IMI Kg^{-1} soil (Table 7.3.6, Fig. 7.3.12).

Table 7.3.6 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on fumarate content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Fumarate content (mg g^{-1} dr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	0.38±0.004	0.40±0.028	0.37±0.002
0	100	0.42±0.010	0.42±0.006	0.38±0.001
250	0	0.40±0.001	0.40±0.018	0.38±0.005
250	100	0.50±0.007	0.41±0.012	0.41±0.031
300	0	0.42±0.004	0.43±0.002	0.39±0.007
300	100	0.51±0.067	0.51±0.007	0.39±0.006
350	0	0.38±0.006	0.41±0.013	0.40±0.003
350	100	0.42±0.004	0.44±0.008	0.42±0.006
Two-way ANOVA				
F_{IMI}		11.0***	27.0***	7.40**
F_{EBR}		48.0***	30.3***	3.93
$F_{IMI \times EBR}$		3.6*	6.8**	1.67
HSD		0.07	0.04	0.03
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Fumarate (30 DAS) = 0.37 + 8.07×10^{-5} IMI + 0.0007 EBR		0.2028	0.6667	0.6983***
Fumarate (60 DAS) = 0.38 + 9.86×10^{-5} IMI + 0.0003 EBR		0.3720	0.4527	0.5860**
Fumarate (90 DAS) = 0.37 + 7.53×10^{-5} IMI + 1×10^{-4} EBR		0.6041	0.2887	0.6696***

*, ** and *** indicate significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. r = multiple correlation coefficient. DAS = days after sowing.

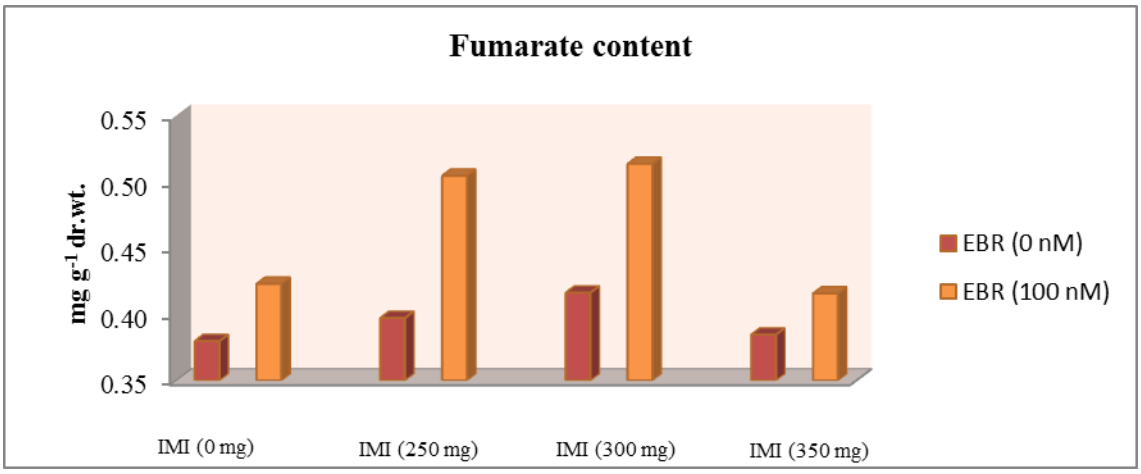

Fig. 7.3.12 Effect of seed soaking with EBR on fumarate content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In 60 days old *B. juncea* plants raised from seeds pre-soaked with 100 nM EBR before sowing in pots containing 300 mg IMI Kg^{-1} soil, fumarate content was increased to 0.51 mg g^{-1} dr. wt. as compared to the control plants in which the fumarate content observed was 0.40 mg g^{-1} dr. wt. (Table 7.3.6, Fig. 7.3.13).

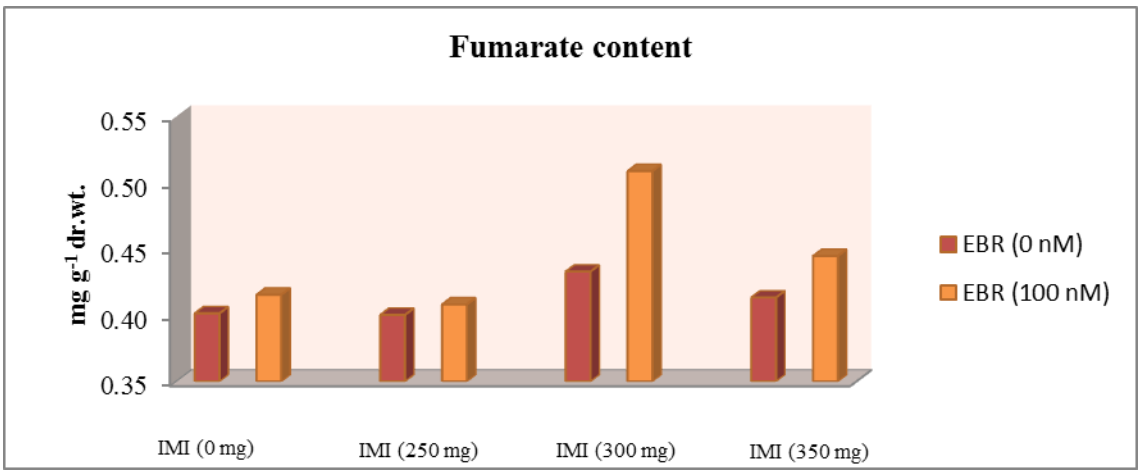

Fig. 7.3.13 Effect of seed soaking with EBR on fumarate content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

Fumarate content in mature control plants (90 DAS) was 0.37 mg g^{-1} dr. wt. and it was increased to 0.42 mg g^{-1} dr. wt. in the leaves of plants raised from EBR (100 nM) treated seeds and grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 7.3.6, Fig. 7.3.14).

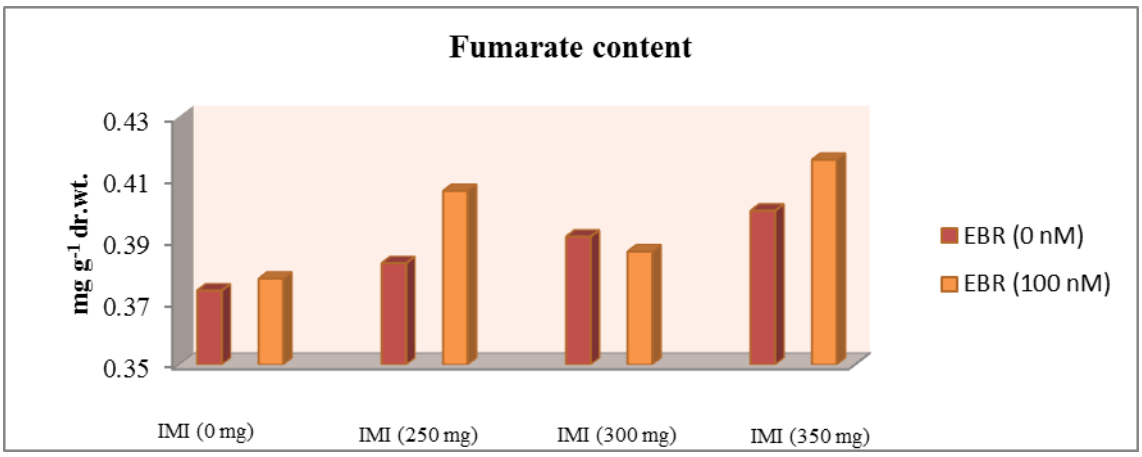

Fig. 7.3.14 Effect of seed soaking with EBR on fumarate content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Significant differences in fumarate contents were observed in *B. juncea* seedlings and leaves after analyzing the data using two-way ANOVA and Tukey's HSD. Analysis of data using MLR revealed that both of IMI as well as EBR applications enhanced the contents of fumarate in *B. juncea* as shown by positive β-regression coefficients (Tables 7.3.5 and 7.3.6). Data analysis using ANN also showed that target and output ascorbate content values were highly correlated (Fig. 7.3.15)

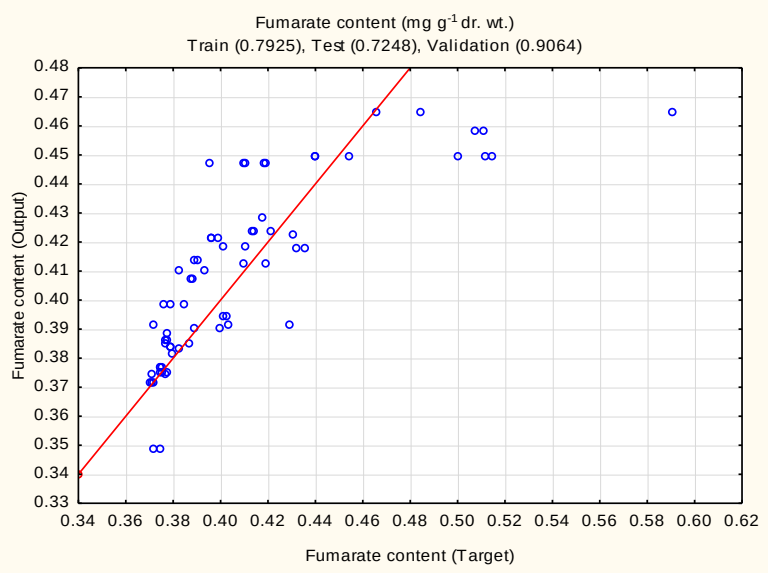

Fig. 7.3.15 Correlation between target (experimental) and output (simulated) fumarate contents using ANN model ($p<0.001$).

Malate

Content of malate in 10 days old control seedlings was 1.39 mg g^{-1} dr. wt. which was maximum increased to 5.94 mg g^{-1} dr. wt. in the seedlings raised from EBR (100 nM) soaked seeds before growing in Petri-plates containing 150 mg IMI L^{-1} (Table 7.3.7, Fig. 7.3.16).

Table 7.3.7 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on malate content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Malate content (mg g^{-1} dr. wt.)		
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	1.39±0.06		
0	100	1.47±0.08		
150	0	2.81±0.31		
150	100	5.94±1.77		
200	0	2.82±0.05		
200	100	5.91±1.03		
250	0	3.29±0.07		
250	100	4.59±0.46		
Two-way ANOVA				
F_{IMI}		21.1***		
F_{EBR}		38.3***		
$F_{IMI \times EBR}$		5.84**		
HSD		2.13		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Malate = 0.87 + 0.0114 IMI + 0.019 EBR		0.5945	0.5320	0.7978***
** and *** indicate significant at p<0.01 and p<0.001 respectively. r = multiple correlation coefficient.				

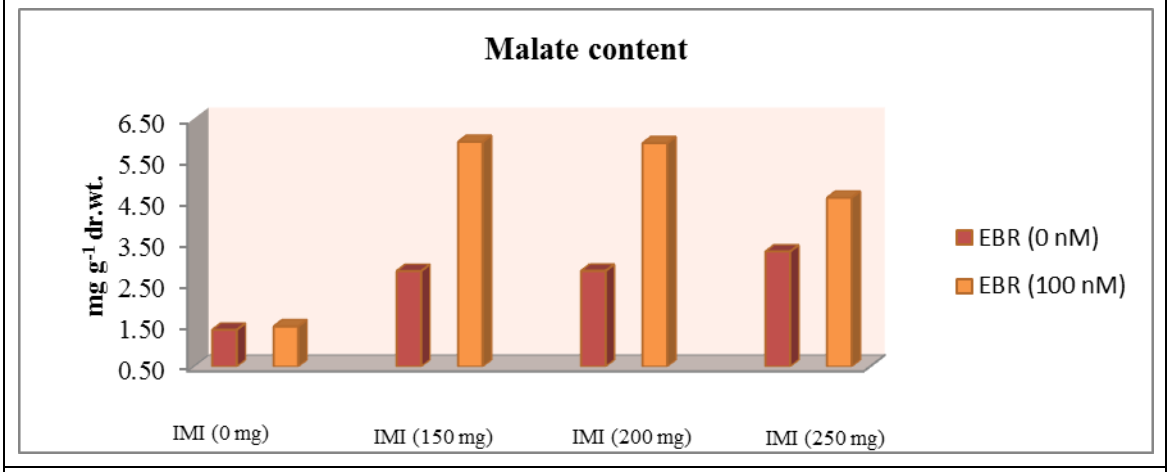

Fig. 7.3.16 Effect of seed soaking with EBR on malate content in *B. juncea* seedlings grown under IMI toxicity.

In the leaves of 30 days old *B. juncea* plants, as compared to control (2.40 mg g^{-1} dr. wt.), maximum malate content observed was 7.19 mg g^{-1} dr. wt. in the leaves of plants germinated from EBR (100 nM) treated seeds and grown in pots amended with 300 mg IMI Kg^{-1} soil (Table 7.3.8, Fig. 7.3.17).

Table 7.3.8 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on malate content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).

Treatments		Malate content (mg g^{-1} dr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	2.40±0.56	14.05±2.77	21.66±3.43
0	100	5.46±1.63	19.45±2.34	20.95±4.80
250	0	3.02±0.47	20.78±3.63	28.44±7.84
250	100	5.14±1.61	28.81±4.66	32.94±12.01
300	0	5.44±1.81	25.93±5.20	35.53±9.56
300	100	7.19±0.87	33.13 ±7.64	44.27±15.38
350	0	2.71±0.81	18.88±0.53	41.76±5.26
350	100	3.04±0.19	39.17±13.75	45.74±7.49
Two-way ANOVA				
F_{IMI}		9.5***	5.2*	7.40**
F_{EBR}		14.9**	15.5**	1.25
$F_{IMI \times EBR}$		1.4	1.7	0.27
HSD		3.2	17.9	25.55
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Malate (30 DAS) = 3.23 + 0.0007 IMI + 0.0182 EBR		0.0507	0.4845	0.4871*
Malate (60 DAS) = 11.55 + 0.0371 IMI + 0.0102 EBR		0.5333	0.5453	0.7628***
Malate (90 DAS) = 18.08 + 0.0612 IMI + 0.0413 EBR		0.7037	0.1763	0.7254***

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

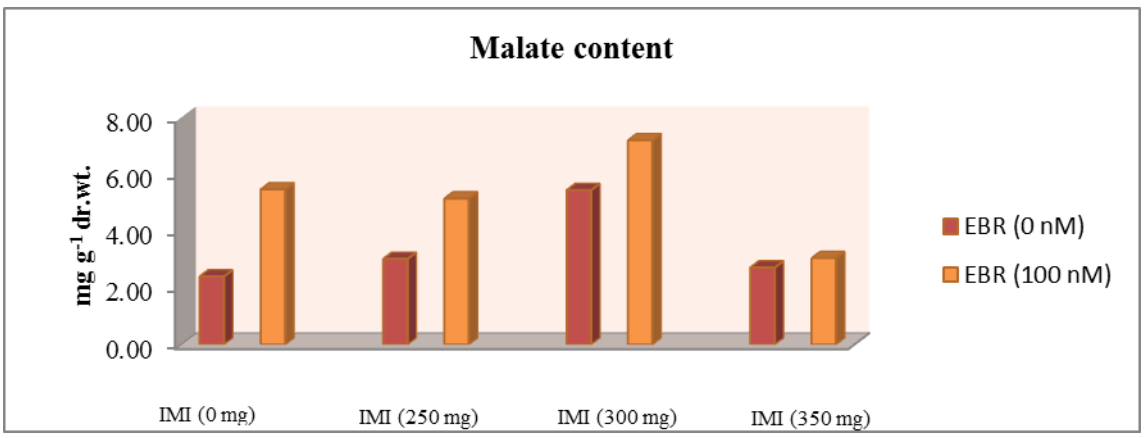

Fig. 7.3.17 Effect of seed soaking with EBR on malate content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In the leaves (60 DAS) of control *B. juncea* plants, malate content observed was 14.05 mg g^{-1} dr. wt. Maximum increase in the content of malate to 39.17 mg g^{-1} dr. wt. was observed in plants raised from EBR (100 nM) treated seeds followed by growing in pots amended with 350 mg IMI Kg^{-1} soil (Table 7.3.8, Fig. 7.3.18).

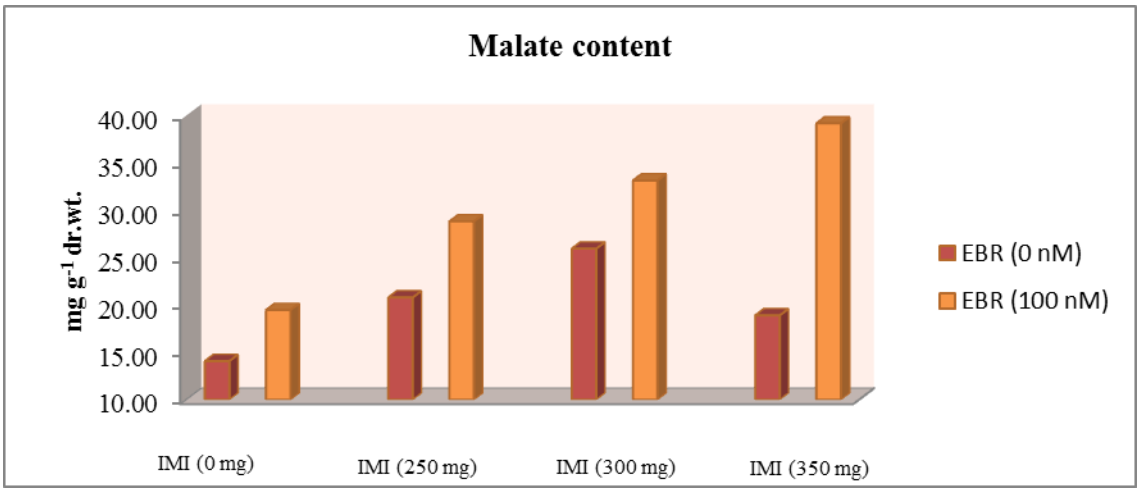

Fig. 7.3.18 Effect of seed soaking with EBR on malate content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

As compared to the leaves of 90 days old control plants (21.66 mg g^{-1} dr. wt.), maximum content of malate (45.74 mg g^{-1} dr. wt.) was observed in plants raised from 100 nM EBR soaked seeds and grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 7.3.8, Fig. 7.3.19).

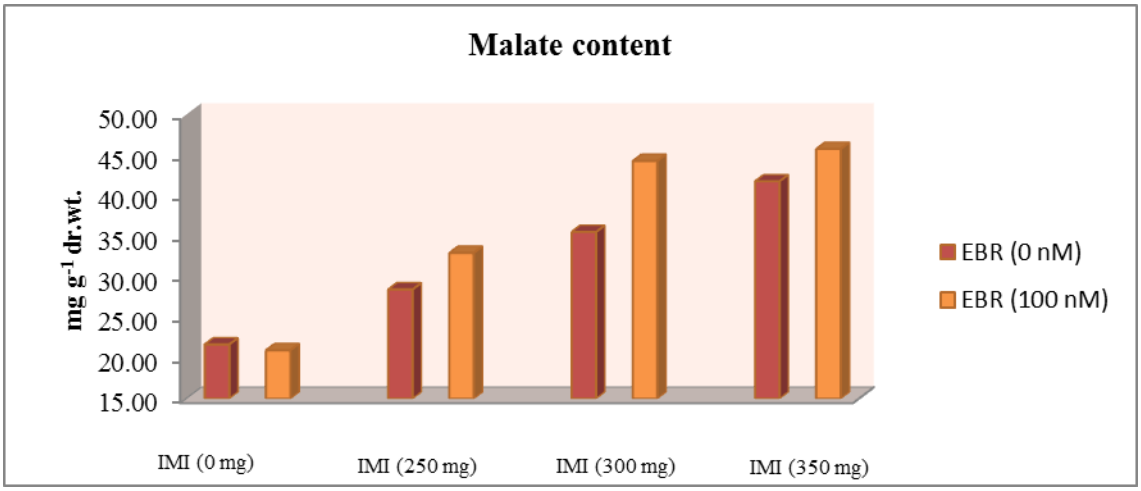

Fig. 7.3.19 Effect of seed soaking with EBR on malate content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

After analysis of data using two-way ANOVA and Tukey's HSD, significant differences in malate contents were observed for *B. juncea* plants given different treatments. Positive values for β-regression obtained from MLR analysis revealed that IMI as well as EBR application significantly enhanced the contents of malate in seedlings and leaves *B. juncea* plants (Tables 7.3.7 and 7.3.8).

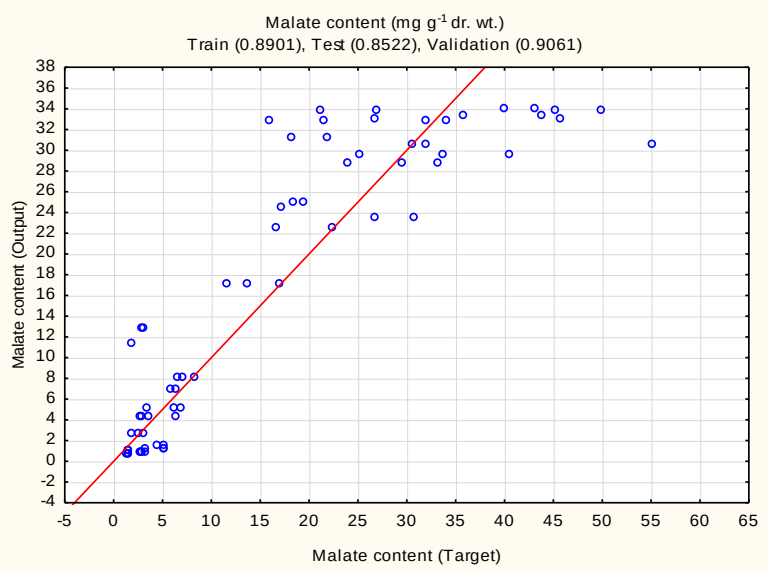

Fig. 7.3.20 Correlation between target (experimental) and output (simulated) malate contents using ANN model (p<0.001).