CHAPTER 5

PIGMENTS AND GASEOUS EXCHANGE PARAMETERS

5.1 Pigments

Chlorophyll content

Chlorophyll contents were observed to decline with the increase in concentration of IMI. As compared to control (301.42 µg g^{-1} fr. wt.), total chlorophyll content in 10 days old *B.* juncea seedlings was maximum decreased to 157.72 µg g^{-1} fr. wt. in the seedlings grown in Petri-plates containing 250 mg L^{-1} IMI. However, seed soaking with 100 nM EBR resulted in recovery of total chlorophyll content which got enhanced to 227.39 µg g^{-1} fr. wt. (Table 5.1.1, Fig. 5.1.1).

Table 5.1.1 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on chlorophyll contents of 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		**Chlorophyll contents (µg g^{-1} fr. wt.)**		
IMI (mg Kg^{-1})	**EBR (nM L^{-1})**	**Chlorophyll-a**	**Chlorophyll-b**	**Total Chlorophyll**
0	0	221.3±2.14	80.15±10.2	301.4±8.58
0	0.1	223.8±2.39	88.23±9.3	311.9±7.10
0	1	215.8±8.48	99.23±1.3	315.0±7.72
0	100	220.7±5.68	111.3±6.9	331.9±6.35
150	0	189.5±19.0	57.50±10.7	247.0±9.17
150	0.1	218.2±7.59	70.07±7.7	288.2±14.8
150	1	223.7±15.2	73.42±24.0	297.1±9.85
150	100	266.0±12.0	87.18±11.3	353.1±23.2
200	0	155.1±12.2	52.31±6.7	207.4±7.91
200	0.1	162.7±6.5	45.30±6.5	208.0±10.3
200	1	163.4±23.0	59.54±14.1	222.9±8.99
200	100	213.2±5.91	67.84±10.8	280.9±5.00
250	0	117.7±6.12	40.05±14.6	157.7±11.8
250	0.1	118.7±6.74	49.73±4.12	168.4±10.3
250	1	146.2±2.87	46.24±6.49	192.4±5.68
250	100	164.9±17.1	62.48±11.3	227.3±26.3
Two-way ANOVA				
F_{IMI}		161.6***	39.87***	281.7***
F_{EBR}		35.3***	11.11***	71.36***
$F_{IMI \times EBR}$		5.65***	0.55	4.26**
HSD		34.3	33.3	37.2

Multiple linear regression

MLR equation	**β-regression coefficients**		**r**
	$β_{IMI}$	**$β_{EBR}$**	
Chl-a = 225.39 - 0.3055 IMI + 0.3674 EBR	- 0.6895	0.3824	0.7885***
Chl-b = 90.97 - 0.1838 IMI + 0.1888 EBR	- 0.8617	0.4081	0.9535***
Total chl = 316.38 - 0.4892 IMI + 0.5560 EBR	- 0.7788	0.4082	0.8793***

** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient.

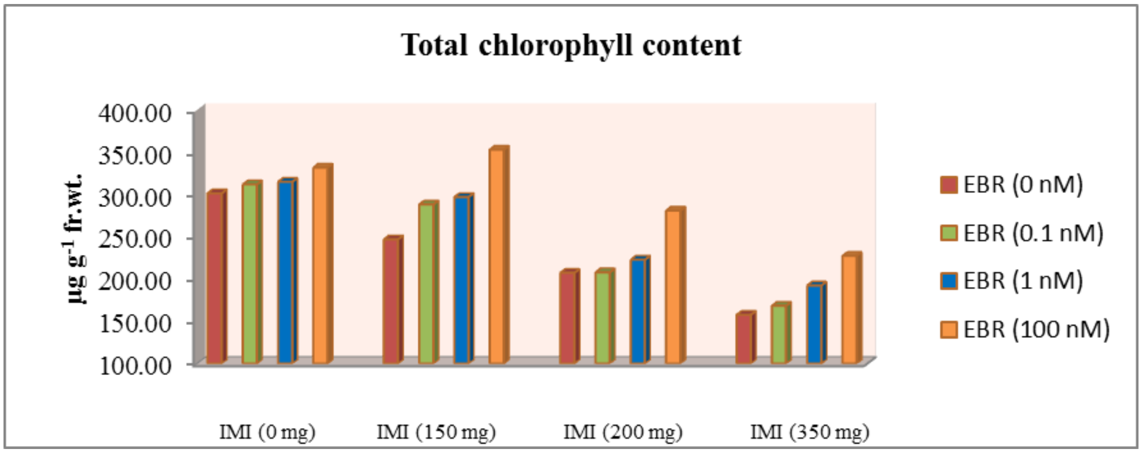

Fig. 5.1.1 Effect of seed soaking with EBR on total chlorophyll content in *B. juncea* seedlings grown under IMI toxicity.

In 30 days old *B. juncea* plants, total chlorophyll content was maximum reduced to 202.0 µg g^{-1} fr. wt. in plants grown in soils amended with 350 mg IMI Kg^{-1} soil, when compared to control plants (322.4 µg g^{-1} fr. wt.). But recovery in total chlorophyll content (256.9 µg g^{-1} fr. wt.) was observed in plants raised from 100 nM EBR treated seeds and grown in pots containing 350 mg IMI Kg^{-1} soil (Table 5.1.2, Fig. 5.1.2).

Table 5.1.2 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on chlorophyll contents 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).

Treatment		Chlorophyll contents (µg g^{-1} fr. wt.)								
IMI	EBR	30 DAS			60 DAS			90 DAS		
(mg Kg^{-1})	(nM L^{-1})	Chl-a	Chl-b	Total chl	Chl-a	Chl-b	Total chl	Chl-a	Chl-b	Total chl
0	0	221.7±0.6	100.8±8.8	322.4±9.3	425.7±10.0	143.7±11.6	569.2±20.1	226.7±0.4	85.4±19.5	312.1±19.2
0	0.1	227.8±4.0	100.9±1.7	328.6±5.4	425.1±11.8	153.7±3.1	578.6±14.7	231.7±2.7	91.5±11.5	323.1±10.3
0	1	222.8±12.1	106.7±13.2	329.4±13.3	445.0±7.6	158.4±7.9	603.2±12.6	229.1±13.4	96.6±15.6	325.5±10.4
0	100	232.9±4.4	108.8±9.5	341.6±12.5	483.5±19.3	137.1±25.8	620.4±16.8	224.5±6.8	122.4±4.4	346.8±11.2
250	0	182.1±22.3	75.2±6.8	257.2±21.8	348.1±8.7	131.0±17.3	479.0±25.1	183.3±21.9	72.1±7.8	255.3±20.6
250	0.1	178.7±11.0	93.4±11.0	272.0±6.1	344.9±9.2	145.9±33.1	490.6±28.7	180.3±11.0	80.0±9.1	260.1±1.8
250	1	187.8±18.2	86.6±10.3	274.2±11.8	385.1±14.4	151.0±5.6	536.0±17.7	212.2±16.8	80.0±2.7	292.1±16.6
250	100	216.5±13.1	72.9±2.0	289.3±13.2	416.4±18.3	157.0±12.0	573.3±10.5	257.7±8.1	83.9±9.5	341.5±12.5
300	0	165.1±3.4	62.7±16.3	227.6±14.0	295.3±10.4	116.0±10.9	411.2±21.1	154.3±6.5	59.3±11.3	213.5±11.0
300	0.1	166.4±3.9	67.1±4.3	233.4±3.3	303.2±6.3	123.3±13.7	426.3±9.2	172.6±11.1	68.4±11.1	240.9±13.2
300	1	172.1±5.5	75.3±7.1	247.3±4.1	320.2±5.7	132.5±7.9	452.5±2.9	184.5±8.1	80.6±14.4	265.1±14.4
300	100	224.7±5.0	68.6±7.4	293.3±12.3	372.9±9.6	136.0±8.2	508.7±1.4	224.1±11.4	90.7±9.0	314.6±13.7
350	0	151.5±5.5	50.6±4.1	202.0±8.5	245.3±25.0	82.4±10.5	327.6±35.4	140.8±5.2	59.9±9.3	200.7±13.3
350	0.1	156.1±4.2	52.2±3.6	208.2±6.2	267.1±8.4	85.6±15.7	352.6±23.9	156.7±6.4	58.1±7.2	214.6±10.5
350	1	170.0±6.9	56.2±6.4	226.1±6.4	285.8±6.0	113.5±8.2	399.2±11.5	178.8±6.7	67.2±6.8	245.9±11.0
350	100	197.6±7.9	59.5±5.8	256.9±12.5	350.5±4.9	120.4±12.5	470.8±17.2	208.0±8.8	78.3±8.4	286.1±16.9
Two-way ANOVA										
F_{IMI}		75.3***	77.2***	202.1***	373.8***	27.3***	262.1***	71.4***	20.8***	98.0***
F_{EBR}		38.3 ***	2.4	33.7***	97.8***	5.2**	61.4***	58.2***	12.0***	72.7***
$F_{IMI \times EBR}$		3.5**	1.41	2.7*	1.8	1.4	2.4*	8.4***	0.9	3.1**
HSD		29.7	25.3	33.6	37.1	44.53	56.4	31.5	32.24	41.2

Multiple linear regression

MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Chl-a (30 DAS) = 218.69 - 0.1570 IMI + 0.3461 EBR	- 0.7727	0.5458	0.9461***
Chl-b (30 DAS) = 106.66 - 0.1305 IMI + 0.0021 EBR	- 0.9196	0.0048	0.9196***
Total chl (30 DAS) = 325.27 - 0.2874 IMI + 0.3482 EBR	- 0.8919	0.3462	0.9567***
Chl-a (60 DAS) = 435.69 - 0.4226 IMI + 0.6540 EBR	- 0.8501	0.4216	0.9489***
Chl-b (60 DAS) = 151.61 - 0.1049 IMI + 0.0973 EBR	- 0.6354	0.1888	0.6629**
Total chl (60 DAS) = 587.16 - 0.5273 IMI + 0.7511 EBR	- 0.8235	0.3758	0.9052***
Chl-a (90 DAS) = 221.28 - 0.1506 IMI + 0.4130 EBR	- 0.6260	0.5502	0.8334***
Chl-b (90 DAS) = 94.87 - 0.0891 IMI + 0.1907 EBR	- 0.7541	0.5175	0.9146***
Total chl (90 DAS) = 316.07 - 0.2395 IMI + 0.6036 EBR	- 0.7061	0.5701	0.9075***

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

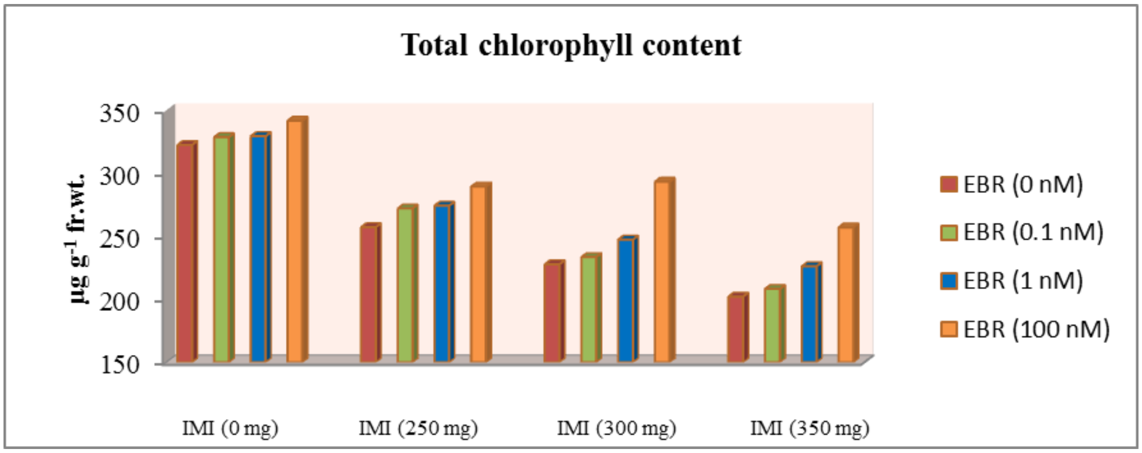

Fig. 5.1.2 Effect of seed soaking with EBR on total chlorophyll content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In plants (60 DAS), grown in soils mixed with 350 mg IMI Kg^{-1} soil, the total chlorophyll content was decreased to 327.6 µg g^{-1} fr. wt. as compared to 569.2 µg g^{-1} fr. wt. in control plants. Further increase in the total chlorophyll content (470.8 µg g^{-1} fr. wt.) was noticed in plants germinated from EBR (100 nM) soaked seeds and grown under IMI toxicity (Table 5.1.2, Fig. 5.1.3).

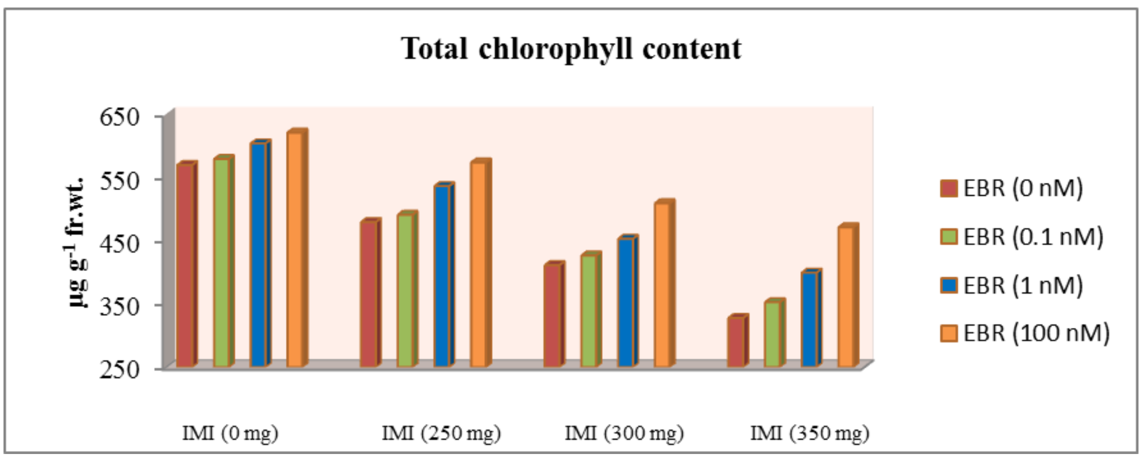

Fig. 5.1.3 Effect of seed soaking with EBR on total chlorophyll content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In 90 days old leaves of *B. juncea* plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil), total chlorophyll content was reduced to 200.7 µg g^{-1} fr. wt. when compared to 312.1 µg g^{-1} fr. wt. in control plants. Seed soaking with 100 nM EBR before sowing resulted in increase in total chlorophyll content to 286.1 μg g^{-1} fr. wt. in plants grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 5.1.2, Fig. 5.1.4).

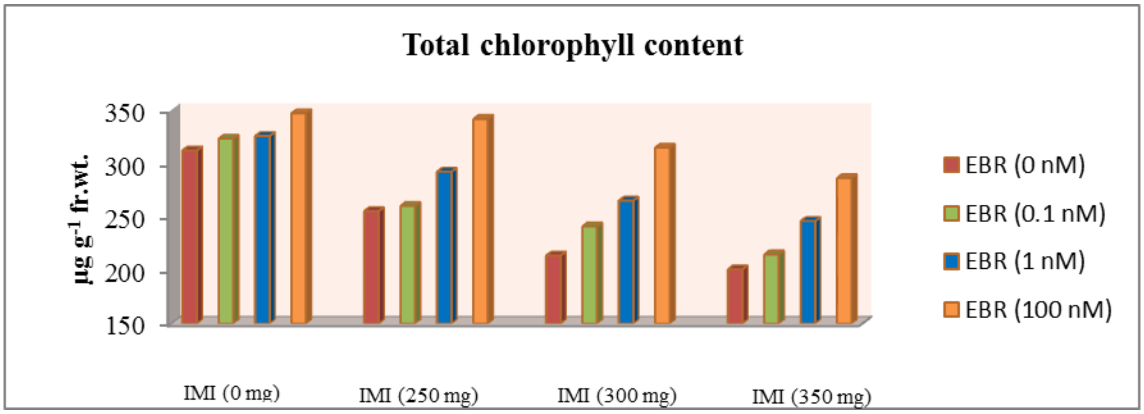

Fig. 5.1.4 Effect of seed soaking with EBR on total chlorophyll content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Two-way ANOVA and Tukey's HSD also showed the significant difference for chlorophyll contents in the seedlings raised from EBR treated seeds and grown under IMI toxicity. MLR analysis also revealed that IMI application reduced the chlorophyll contents as indicated by negative β-regression coefficients, whereas EBR seed soaking enhanced the chlorophyll contents (positive β-regression coefficients) as shown in table 5.1.1 and 5.1.2. High correlation was obtained in between simulated and experimental values using ANN model (Fig. 5.1.5)

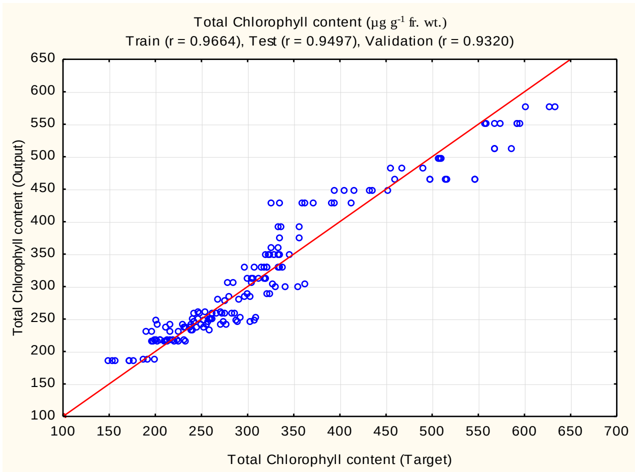

Fig. 5.1.5 Correlation between target (experimental) and output (simulated) total chlorophyll contents using ANN model (p<0.001).

Carotenoid content

As compared to controls, carotenoid content in *B. juncea* was increased under IMI toxicity. In 10 days old seedlings, maximum enhancement in the carotenoid content observed was 120.2 µg g^{-1} fr. wt. in seedlings grown in Petri-plates containing 200 mg IMI L^{-1} when compared to the control seedlings (86.29 µg g^{-1} fr. wt.). Seed soaking with EBR (100 nM) also enhanced the carotenoid content to 173.5 µg g^{-1} fr. wt. in seedlings grown under IMI toxicity (Table 5.1.3, Fig. 5.1.6).

Table 5.1.3 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on carotenoid content of 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			Carotenoid content (µg g^{-1} fr. wt.)	
IMI (mg L^{-1})	EBR (nM L^{-1})			
0	0	86.29±1.72		
0	0.1	90.05±2.33		
0	1	91.98±5.46		
0	100	107.7±6.53		
150	0	99.14±7.85		
150	0.1	103.3±2.65		
150	1	111.2±6.33		
150	100	146.9±13.7		
200	0	120.2±13.3		
200	0.1	122.4±6.99		
200	1	127.1±4.22		
200	100	173.5±13.3		
250	0	102.1±3.58		
250	0.1	121.6±8.17		
250	1	126.3±5.01		
250	100	146.2±10.7		
Two-way ANOVA				
F_{IMI}		58.52***		
F_{EBR}		62.63***		
$F_{IMI \times EBR}$		3.06**		
HSD		24.2		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		$β_{IMI}$	**$β_{EBR}$**	
Caro = 86.50 + 0.1456 IMI + 0.3534 EBR		0.5967	0.6679	0.8956***
** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient.				

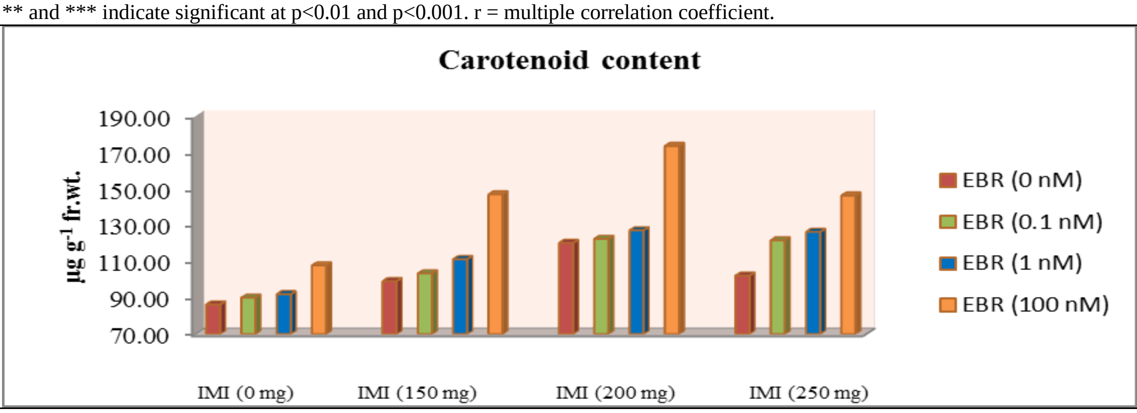

Fig. 5.1.6 Effect of seed soaking with EBR on carotenoid content in *B. juncea* seedlings grown under IMI toxicity.

In 30 days old plants grown under IMI stress (300 mg IMI Kg^{-1} soil), maximum carotenoid content observed was 170.9 µg g^{-1} fr. wt. in comparison to the control plants (110.2 µg g^{-1} fr. wt.). Further enhancement in carotenoid content was noticed (224.3 µg g^{-1} fr. wt.) in plants germinated from 100 nM EBR soaked seeds and grown in soils mixed with 300 mg IMI Kg^{-1} soil (Table 5.1.4, Fig. 5.1.7).

Table 5.1.4 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on carotenoid 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		Carotenoid content (µg g^{-1} fr. wt.)		
IMI (mg Kg^{-1})	**EBR (nM L^{-1})**	**30 DAS**	**60 DAS**	**90 DAS**
0	0	110.2±10.3	166.1±6.5	153.6±7.3
0	0.1	120.3±2.9	172.7±5.9	156.5±5.1
0	1	121.6±13.4	176.7±6.8	161.5±5.2
0	100	138.8±6.2	191.5±3.8	163.7±8.7
250	0	138.9±7.5	188.5±1.3	163.9±7.1
250	0.1	140.1±6.7	194.8±16.4	178.3±5.7
250	1	142.5±24.1	200.6±7.1	194.8±16.4
250	100	162.5±11.1	232.2±16.7	201.9±6.1
300	0	170.9±1.9	205.1±2.5	185.4±11.2
300	0.1	172.6±3.4	226.3±1.6	193.8±7.1
300	1	175.8±4.1	237.7±15.3	204.6±5.5
300	100	224.3±16.1	271.6±16.2	223.6±11.6
350	0	148.3±4.2	180.6±14.8	166.3±3.6
350	0.1	158.1±5.5	186.4±10.8	175.6±8.1
350	1	168.6±8.3	200.8±14.2	172.2±5.3
350	100	190.6±16.1	232.3±10.7	192.3±7.5
Two-way ANOVA				
F_{IMI}		77.8***	57.9***	56.5***
F_{EBR}		28.4***	40.7***	24.9***
$F_{IMI \times EBR}$		1.5	1.5	2.1
HSD		32.2	33.1	24.9

Multiple linear regression

MLR equation	β-regression coefficients β_{IMI}	β-regression coefficients β_{EBR}	r
Caro (30 DAS) = 113.78 + 0.1487 IMI + 0.3192 EBR	0.7136	0.4907	0.8660***
Caro (60 DAS)= 169.81 + 0.1099 IMI + 0.3747 EBR	0.5334	0.5824	0.7898***
Caro (90 DAS) = 156.56 + 0.0840 IMI + 0.2004 EBR	0.5862	0.4479	0.7377**

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

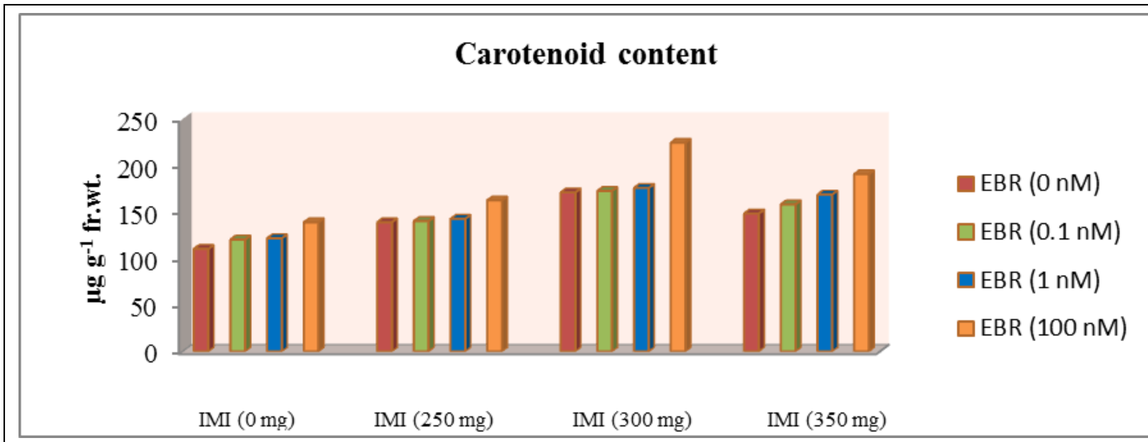

Fig. 5.1.7 Effect of seed soaking with EBR on carotenoid content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Carotenoid content in 60 days old plants grown under IMI toxicity (300 mg IMI Kg^{-1} soil) was maximum increased to 205.1 µg g^{-1} fr. wt. as compared to 166.1 µg g^{-1} fr. wt. in control plants. EBR (100 nM) seed treatment further enhanced the carotenoid content to 271.6 in *B. juncea* plants grown in soils mixed with 300 mg IMI Kg^{-1} soil (Table 5.1.4, Fig. 5.1.8).

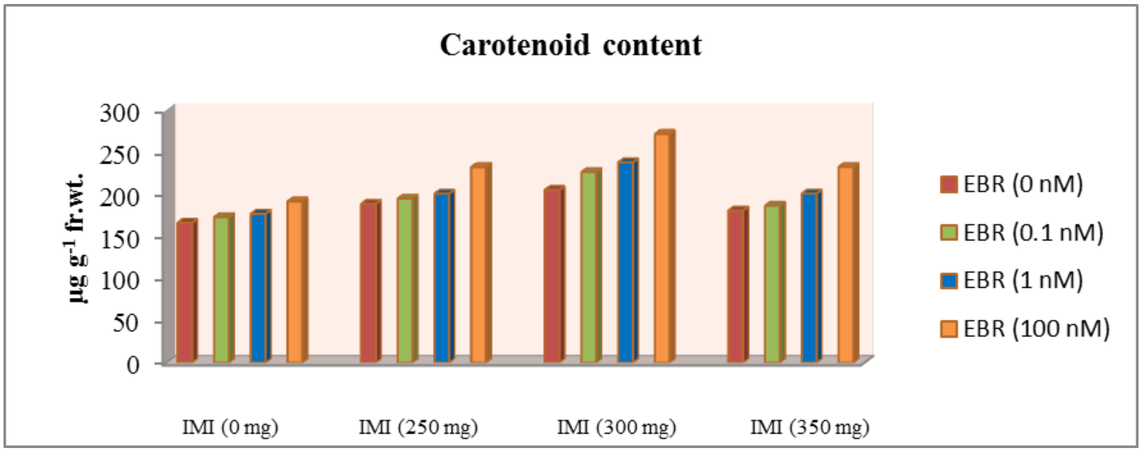

Fig. 5.1.8 Effect of seed soaking with EBR on carotenoid content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In 90 days old plants, as compared to control (153.6 µg g^{-1} fr. wt.), carotenoid content was increased to 185.4 µg g^{-1} fr. wt. in plants grown under IMI toxicity (300 mg IMI Kg^{-1} soil). Further increase in carotenoid content was noticed in plants raised from 100 nM EBR soaked seeds and grown in pots containing 300 mg IMI Kg^{-1} soil (Table 5.1.4, Fig. 5.1.9).

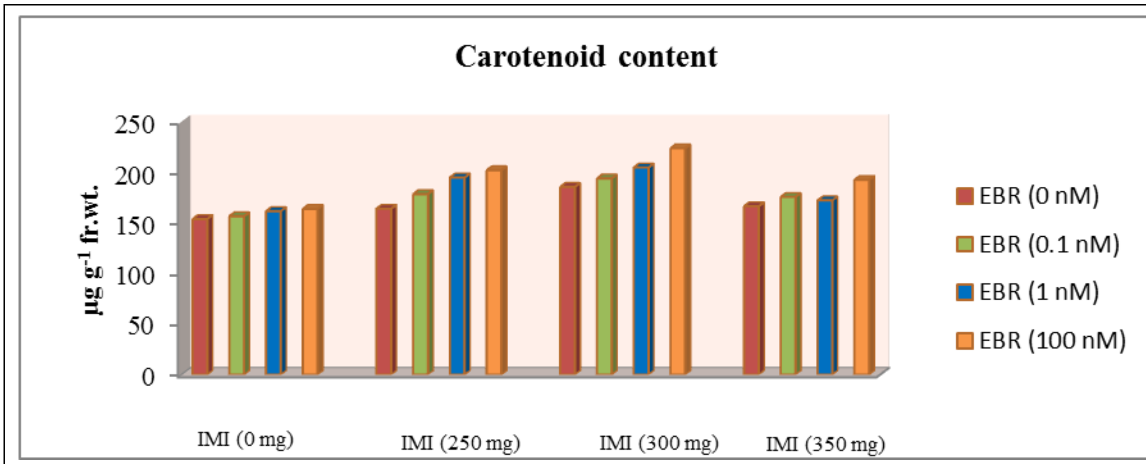

Fig. 5.1.9 Effect of seed soaking with EBR on carotenoid content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Data analysis using two-way ANOVA and Tukey's HSD revealed the significant difference for carotenoid content. MLR analysis showed that both IMI as well as EBR application enhanced the carotenoid content in *B. juncea* as indicated by positive β-regression coefficients (Table 5.1.3, 5.1.4). Analysis of data using ANN showed that model can simulate the data at very high level of significance ($p<0.001$) as shown in Fig. 5.1.10.

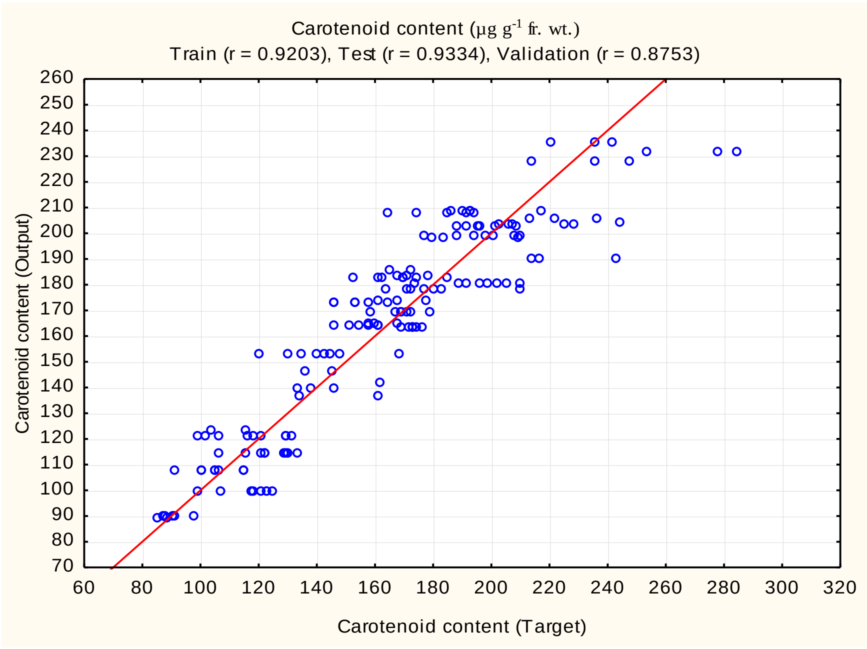

Fig. 5.1.10 Correlation between target (experimental) and output (simulated) carotenoid contents using ANN model (p<0.001).

Anthocyanin content

Anthocyanin content was noticed to increase in both IMI and EBR treatment treatments. In 10 days old seedlings, in comparison to control (13.0 µg g^{-1} fr. wt.) anthocyanin content was maximum increased to 33.5 µg g^{-1} fr. wt. in seedlings raised from 100 nM EBR treated seeds and grown in Petri-plates supplemented with 200 mg IMI L^{-1} (Table 5.1.5, Fig. 5.1.11).

Table 5.1.5 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on anthocyanin content of 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			Anthocyanin content ($\mu g\ g^{-1}$ fr. wt.)	
IMI ($mg\ Kg^{-1}$)	EBR ($nM\ L^{-1}$)			
0	0	13.03±0.96		
0	0.1	14.72±0.73		
0	1	15.21±0.84		
0	100	17.82±0.61		
150	0	13.71±1.89		
150	0.1	15.30±0.46		
150	1	16.62±0.96		
150	100	21.31±1.71		
200	0	18.73±0.76		
200	0.1	21.10±1.08		
200	1	24.33±0.87		
200	100	33.56±0.85		
250	0	14.48±1.11		
250	0.1	15.09±1.77		
250	1	17.13±0.70		
250	100	21.62±1.72		
Two-way ANOVA				
F_{IMI}		153.5***		
F_{EBR}		125.7***		
$F_{IMI \times EBR}$		8.69***		
HSD		3.5		
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		β_{IMI}	β_{EBR}	
Anth = 13.80 + 0.0186 IMI + 0.0700 EBR		0.3445	0.5993	0.6914**
** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient.				

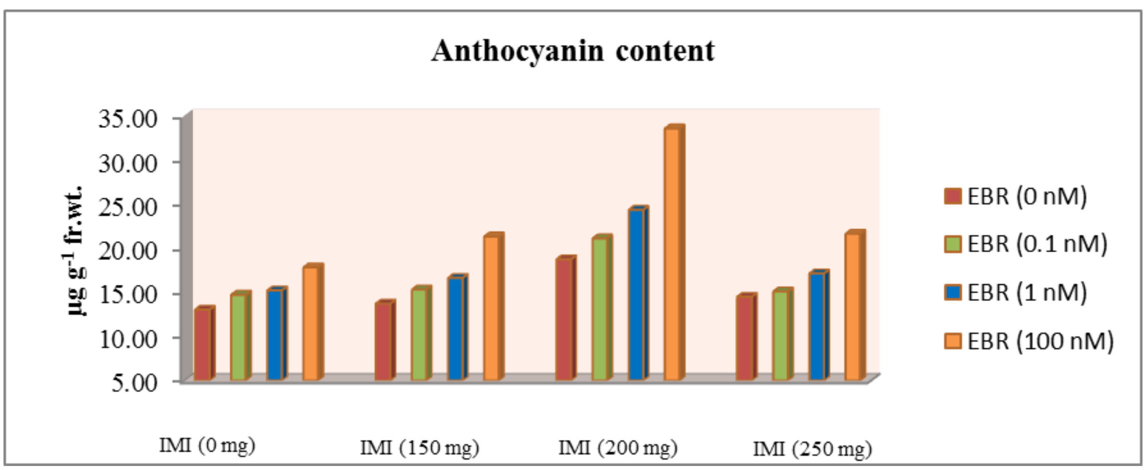

Fig. 5.1.11 Effect of seed soaking with EBR on anthocyanin content in *B. juncea* seedlings grown under IMI toxicity.

In 30 days old *B. juncea* plants, anthocyanin content observed was 15.1 µg g^{-1} fr. wt. in control plants which was increased to 24.3 µg g^{-1} fr. wt. in the leaves of plants raised from 100 nM EBR treated seeds and grown in soils amended with 300 mg IMI Kg^{-1} soil (Table 5.1.6, Fig. 5.1.12).

Table 5.1.6 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on anthocyanin 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		Anthocyanin content (µg g^{-1} fr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	15.1±0.4	19.1±0.8	23.5±1.1
0	0.1	15.5±0.2	19.9±0.5	24.1±1.1
0	1	15.6±0.4	20.1±0.3	23.8±1.1
0	100	17.1±1.5	20.2±1.1	25.9±1.2
250	0	17.6±1.4	20.1±0.7	24.7±1.2
250	0.1	18.2±1.9	20.5±0.4	24.9±0.5
250	1	19.1±1.7	21.5±0.8	25.5±1.8
250	100	21.1±2.1	23.9±0.8	28.1±0.3
300	0	18.8±1.7	21.2±0.6	26.6±0.4
300	0.1	19.4±1.4	22.6±0.5	27.7±1.1
300	1	19.8±1.0	23.6±0.7	28.1±1.5
300	100	24.3±1.8	27.1±2.2	30.1±0.4
350	0	17.8±0.5	19.6±0.9	24.1±1.7
350	0.1	18.9±1.5	19.8±0.6	24.9±1.5
350	1	19.6±1.7	21.5±0.7	26.3±2.7
350	100	22.5±1.1	23.3±0.8	28.4±3.2
Two-way ANOVA				
F_{IMI}		25.7***	35.2***	11.9***
F_{EBR}		17.3***	35.1***	10.5***
$F_{IMI \times EBR}$		0.7	2.8*	0.2
HSD		4.3	2.7	4.6

Multiple linear regression

MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Anth (30 DAS) = 15.08 + 0.0128 IMI + 0.0331 EBR	0.7172	0.5943	0.9315***
Anth (60 DAS) = 19.32 + 0.0066 IMI + 0.0285 EBR	0.4372	0.6018	0.7438**
Anth (90 DAS) = 23.78 + 0.0070 IMI + 0.0276 EBR	0.5101	0.6368	0.8159***

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

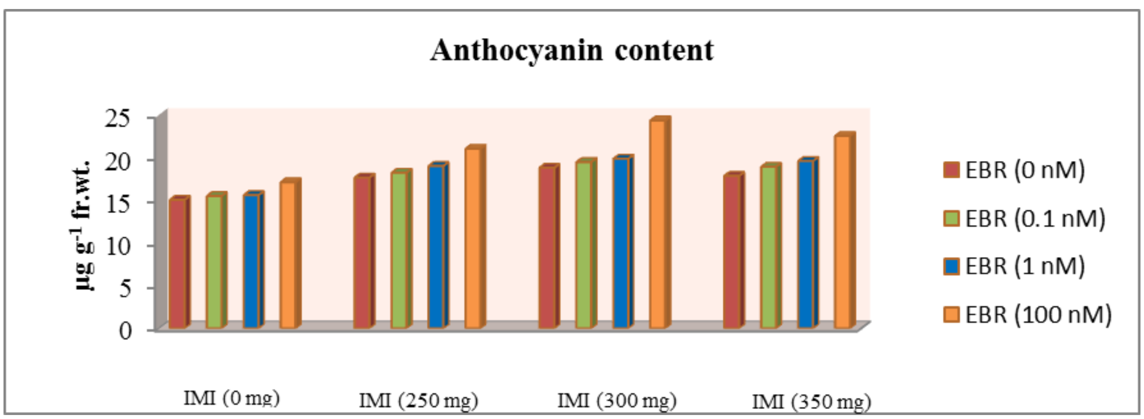

Fig. 5.1.12 Effect of seed soaking with EBR on anthocyanin content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Anthocyanin content noticed in the leaves of 60 days old control plants was 19.1 µg g^{-1} fr. wt. which was further enhanced to 27.1 µg g^{-1} fr. wt. in the leaves of *B. juncea* plants germinated from EBR (100 nM) soaked seeds and grown in pots containing 300 mg IMI Kg^{-1} soil (Table 5.1.6, Fig. 5.1.13).

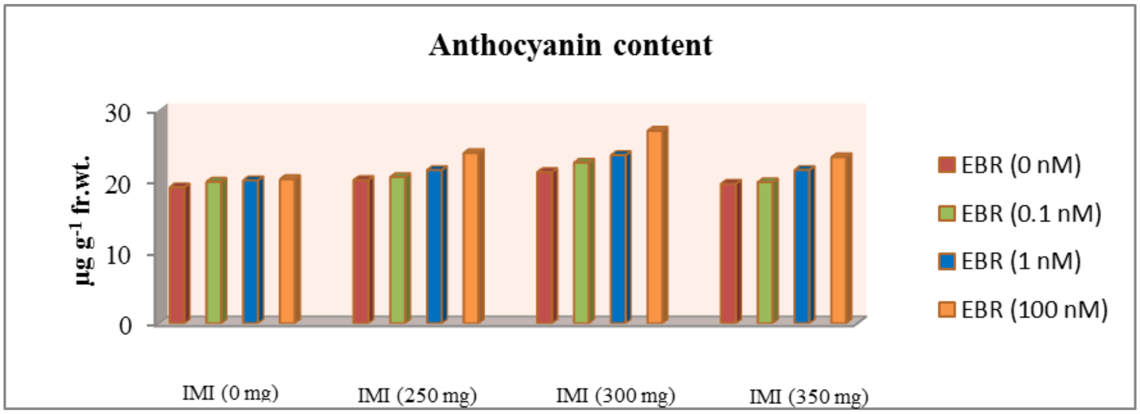

Fig. 5.1.13 Effect of seed soaking with EBR on anthocyanin content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In control plants (90 DAS), anthocyanin content observed was 23.5 µg g^{-1} fr. wt. which was increased to 30.1 µg g^{-1} fr. wt. in plants raised from EBR (100 nM) treated seeds and grown in soils containing 300 mg IMI Kg^{-1} soil (Table 5.1.6, Fig. 5.1.14).

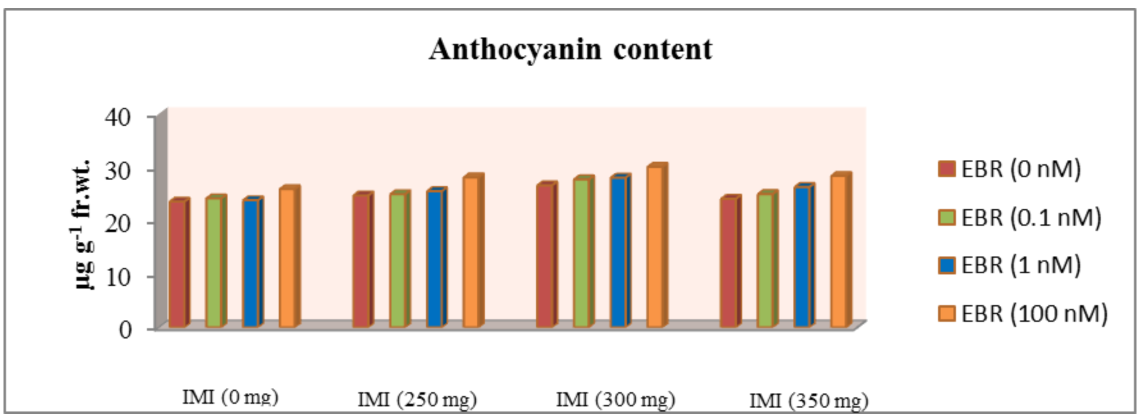

Fig. 5.1.14 Effect of seed soaking with EBR on anthocyanin content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Two-way ANOVA and Tukey's HSD showed significant differences in anthocyanin contents in seedlings raised from both EBR treated as well as untreated seeds and grown under IMI toxicity. MLR analysis of data also showed that both IMI as well as EBR treatments increased the anthocyanin content in *B. juncea* as observed from positive β-regression values (Tables 5.1.5 and 5.1.6). High correlation was observed between target and output values after analyzing the data using ANN (Fig. 5.1.15)

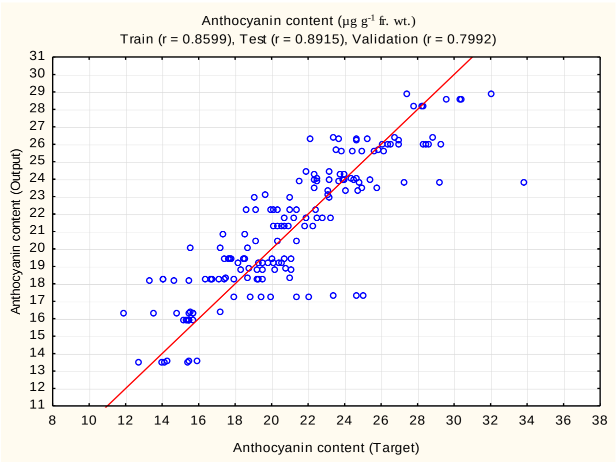

Fig. 5.1.15 Correlation between target (experimental) and output (simulated) anthocyanin contents using ANN model (p<0.001).

Xanthophyll content

Xanthophyll content was noticed to increase in the seedlings and plants *B. juncea* with both of EBR as well as IMI treatment. In 10 days old seedlings (control), xanthophyll content noticed was 8.99 mg g^{-1} dr. wt. which was further enhanced to 35.6 mg g^{-1} dr. wt. in seedlings raised from 100 nM EBR soaked seeds and grown in IMI (200 mg L^{-1}) containing Petri-plates (Table 5.1.7, Fig. 5.1.16).

Table 5.1.7 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on xanthophyll content of 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		Xanthophyll content (mg g^{-1} dr. wt.)
IMI (mg Kg^{-1})	EBR (nM L^{-1})	
0	0	8.99±0.59
0	0.1	9.60±0.99
0	1	10.17±0.65
0	100	11.49±0.78
150	0	12.90±0.83
150	0.1	13.47±0.36
150	1	15.63±1.27
150	100	18.22±0.99
200	0	19.96±4.24
200	0.1	20.06±1.94
200	1	22.46±2.22
200	100	35.69±4.23
250	0	16.62±1.07
250	0.1	17.47±1.98
250	1	20.90±2.50
250	100	26.18±0.94
Two-way ANOVA		
F_{IMI}		121.4***
F_{EBR}		43.97***
$F_{IMI \times EBR}$		6.41***
HSD		5.9

Multiple linear regression			
MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Xanth = 8.17 + 0.0499 IMI + 0.0725 EBR	0.6951	0.4660	0.8369***

*** indicates significant at p<0.001. r = multiple correlation coefficient.

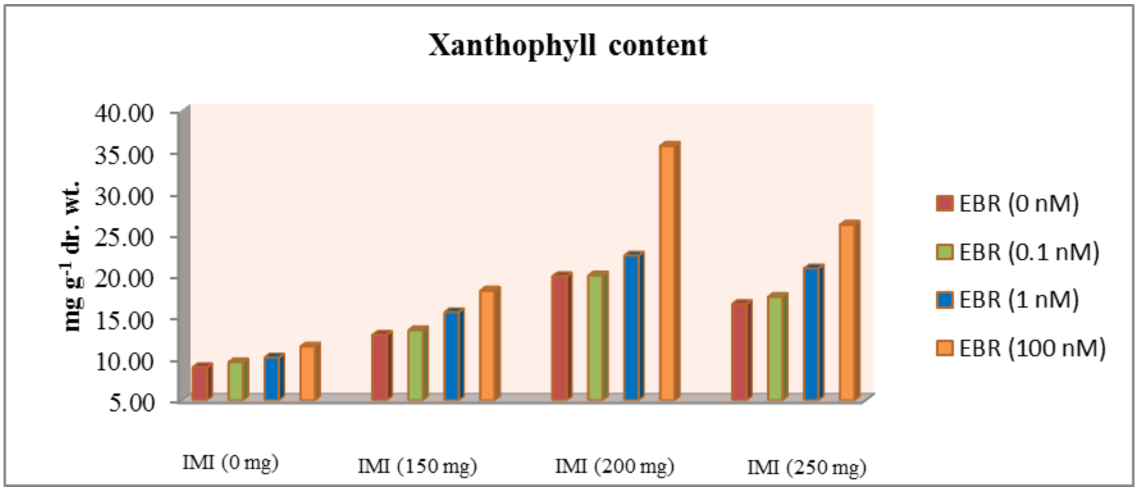

Fig. 5.1.16 Effect of seed soaking with EBR on xanthophyll content in *B. juncea* seedlings grown under IMI toxicity.

In 30 days old control plants of *B. juncea*, xanthophyll content observed was 12.7 mg g^{-1} dr. wt. which was increased to 23.2 mg g^{-1} dr. wt. in the leaves of plants which were germinated from EBR (100 nM) soaked seeds and grown in soils supplemented with 350 mg IMI Kg^{-1} soil (Table 5.1.8, Fig. 5.1.17).

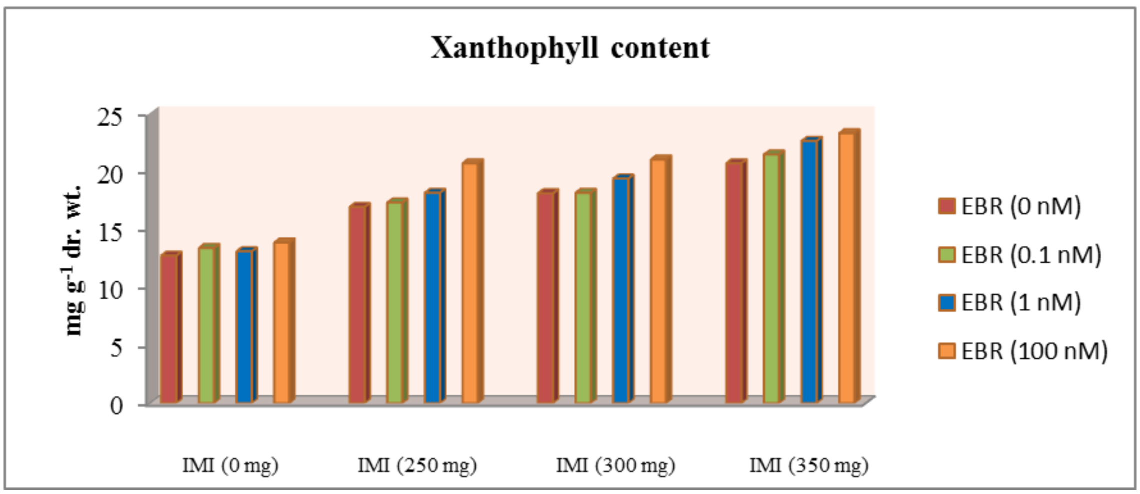

Fig. 5.1.17 Effect of seed soaking with EBR on xanthophyll content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Xanthophyll content noticed in 60 days old control plants was 15.6 mg g^{-1} dr. wt. which was further increased to 24.1 mg g^{-1} dr. wt. in plants raised from seeds treated with EBR (100 nM) and grown in pots containing 350 mg IMI Kg^{-1} soil (Table 5.1.8, Fig. 5.1.18).

Table 5.1.8 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on xanthophyll 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		Xanthophyll content (mg g^{-1} dr. wt.)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	12.7±0.6	15.6±0.6	19.3±0.9
0	0.1	13.3±1.1	15.9±1.3	19.4±1.1
0	1	13.1±0.6	16.2±1.5	20.2±0.6
0	100	13.8±0.7	16.3±1.7	21.7±0.7
250	0	16.9±0.9	16.6±1.4	20.2±0.5
250	0.1	17.3±1.2	17.8±0.9	20.7±0.8
250	1	18.1±0.8	17.9±1.1	21.8±1.2
250	100	20.6±0.8	19.2±1.9	23.1±1.1
300	0	18.1±0.5	19.3±1.1	21.1±1.8
300	0.1	18.1±1.0	19.7±1.1	21.8±2.2
300	1	19.3±1.0	20.3±1.2	22.0±1.6
300	100	20.9±1.3	23.3±1.6	27.2±0.8
350	0	20.6±1.1	20.5±0.9	21.3±2.4
350	0.1	21.4±1.1	21.2±2.2	21.5±1.5
350	1	22.5±0.8	20.6±0.4	21.7±0.5
350	100	23.2±0.8	24.1±2.1	24.9±1.5
Two-way ANOVA				
F_{IMI}		178.6***	37.2***	9.9***
F_{EBR}		17.4***	7.6***	18.1***
$F_{IMI \times EBR}$		1.3	0.8	1.2
HSD		2.8	4.3	4.1

Multiple linear regression

MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Xanth (30 DAS) = 12.48 + 0.0228 IMI + 0.0206 EBR	0.9236	0.2671	0.9615***
Xanth (60 DAS) = 19.30 + 0.0073 IMI + 0.0331 EBR	0.5082	0.7371	0.8953***
Xanth (90 DAS) = 15.12 + 0.0150 IMI + 0.0223 EBR	0.8107	0.3854	0.8977***

*** indicates significant at p<0.001. r = multiple correlation coefficient. DAS = days after sowing.

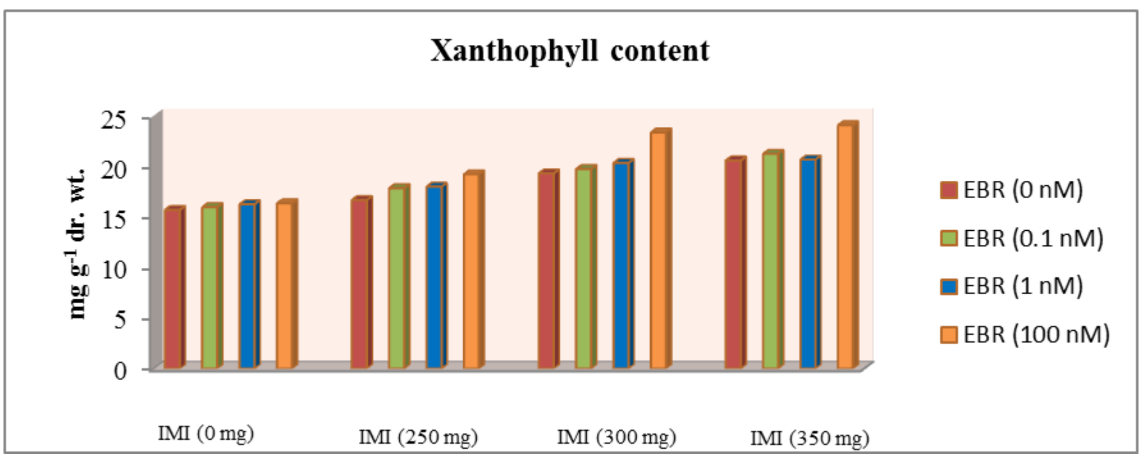

Fig. 5.1.18 Effect of seed soaking with EBR on xanthophyll content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

In 90 days old control plants, xanthophyll content measured was 19.3 mg g^{-1} dr. wt. In plants raised from seeds which were soaked in 100 nM EBR and germinated in soils amended with 300 mg IMI Kg^{-1} soil, xanthophyll content was increased to 27.2 mg g^{-1} dr. wt. as compared to control plants (Table 5.1.8, Fig. 5.1.19).

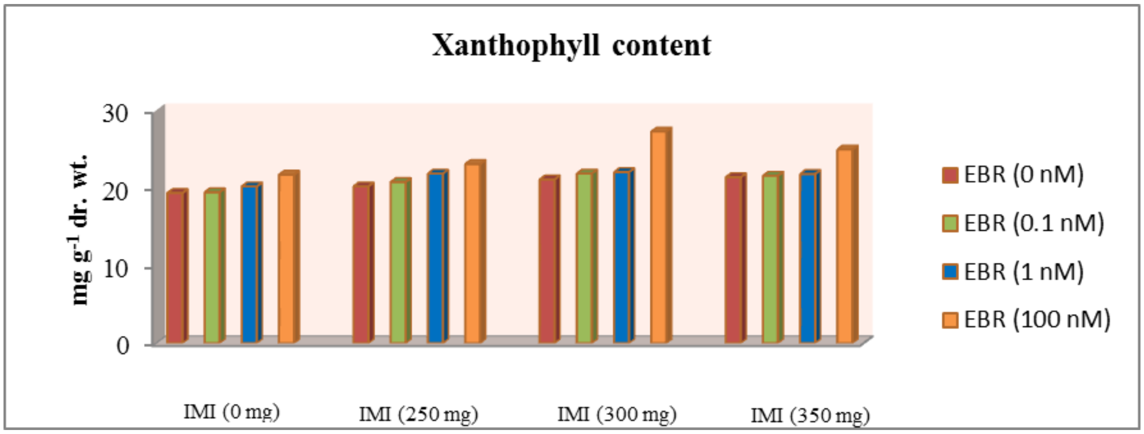

Fig. 5.1.19 Effect of seed soaking with EBR on xanthophyll content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Significant difference in xanthophyll contents was observed after data analysis using two-way ANOVA and Tukey's HSD. Positive β-regression coefficients obtained from MLR analysis also revealed the enhanced xanthophyll contents with both of IMI and EBR treatment (Tables 5.1.6 and 5.1.7). Data analysis using ANN model showed that simulated (output) and experimental (target) values were highly correlated (Fig. 5.1.20).

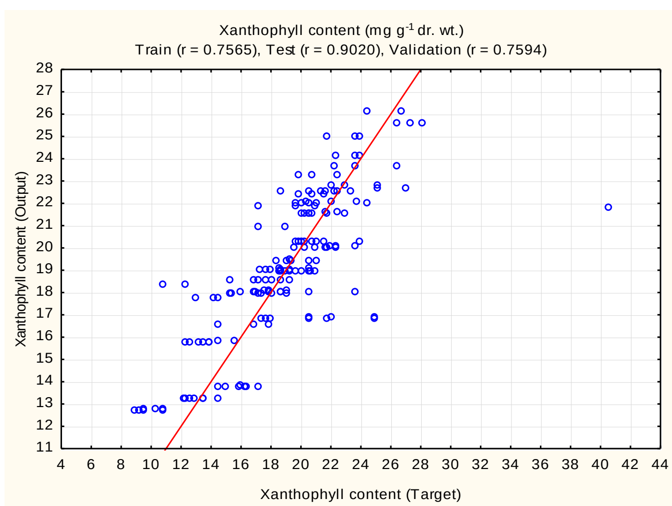

Fig. 5.1.20 Correlation between target (experimental) and output (simulated) xanthophyll contents using ANN model (p<0.001).

5.2 Gaseous exchange parameters

Net photosynthetic rate (Pn)

As compared to control plants, Pn was observed to decrease with the increase in IMI concentration in soil. In 30 days old plants, the maximum decrease in Pn (33.7 µmole CO_2 m^{-2} s^{-1}) was observed at 350 mg IMI Kg^{-1} soil as compared to the control plants (Pn = 50.9 µmole CO_2 m^{-2} s^{-1}). Moreover, seed soaking with 100 nM EBR enhanced the Pn to 45.8 µmole CO_2 m^{-2} s^{-1} in plants grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 5.2.1, Fig. 5.2.1).

Table 5.2.1 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on net photosynthetic rate 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		Net photosynthetic rate (Pn) (µmole CO_2 m^{-2} s^{-1})		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	50.9 ± 0.7	65.2 ± 1.1	47.9 ± 0.1
0	0.1	53.4 ± 0.6	68.2± 0.5	49.1 ± 0.2
0	1	55.4 ± 0.3	72.2 ± 0.3	48.2 ± 0.7
0	100	57.3 ± 1.3	73.9 ± 0.5	51.1 ± 1.2
250	0	46.7 ± 1.7	52.3 ± 1.5	46.0 ± 0.4
250	0.1	49.0 ± 1.1	55.5 ± 1.6	46.9 ± 0.9
250	1	54.6 ± 0.8	62.2 ± 0.5	51.0 ± 1.1
250	100	60.3 ± 1.8	70.7 ± 0.3	53.7 ± 3.5
300	0	41.9 ± 1.8	45.8 ± 0.2	37.4 ± 0.5
300	0.1	45.9 ± 1.6	50.6 ± 0.5	46.3 ± 0.9
300	1	48.2 ± 0.4	52.8 ± 1.1	47.0 ± 0.1
300	100	54.6 ± 0.9	65.8 ± 0.4	48.8 ± 1.2
350	0	33.7 ± 0.2	42.9 ± 1.5	29.3 ± 0.2
350	0.1	42.7 ± 0.4	46.1 ± 0.4	37.1 ± 1.2
350	1	44.3 ± 0.9	50.6 ± 0.7	31.8 ± 1.7
350	100	45.8 ± 1.5	55.5 ± 0.4	41.2 ± 4.2
Two way ANOVA				
F_{IMI}		279.1***	1305.6***	213.2***
F_{EBR}		191.7***	652.7***	56.3***
$F_{IMI \times EBR}$		9.4***	26.0***	7.9***
HSD		3.5	2.6	4.8
Multiple linear regression				
MLR equation		**β-regression coefficients**		**MLR r**
		$β_{IMI}$	**$β_{EBR}$**	
Pn (30 DAS) = 53.78 - 0.0291 IMI + 0.0735 EBR		- 0.5955	0.4817	0.7659***
Pn (60 DAS) = 68.01 - 0.0563 IMI + 0.1122 EBR		- 0.7708	0.4921	0.9145***
Pn (90 DAS) = 49.66 - 0.0288 IMI + 0.0557 EBR		- 0.5618	0.3477	0.6607**

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

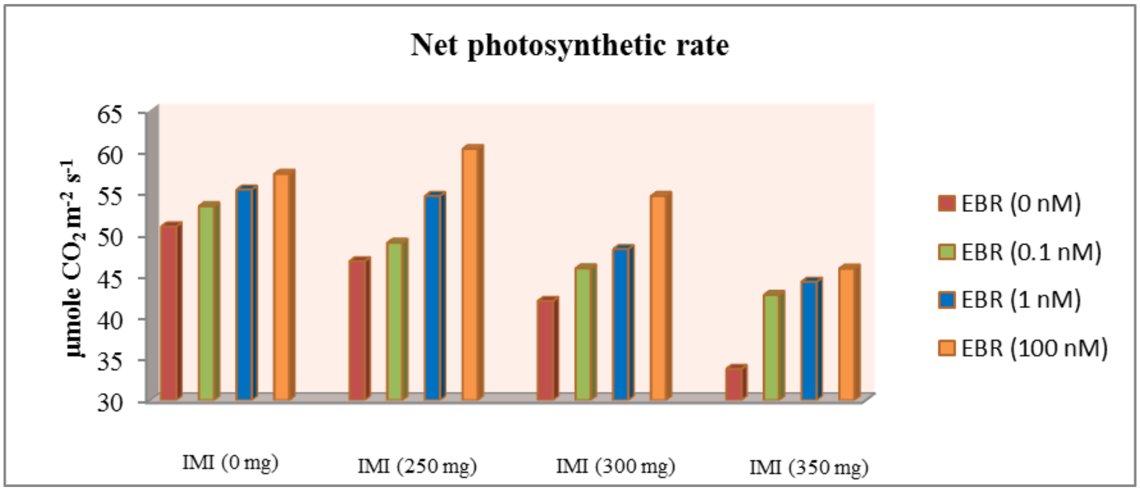

Fig. 5.2.1 Effect of seed soaking with EBR on net photosynthetic rate in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In 60 days old plants grown in soils amended with 350 mg IMI Kg^{-1} soil, Pn was maximum decreased to 42.9 µmole CO_2 m^{-2} s^{-1} when compared to Pn of control plants (65.2 µmole CO_2 m^{-2} s^{-1}). Additionally, seed soaking with 100 nM EBR resulted in increase in Pn to 55.5 µmole CO_2 m^{-2} s^{-1} under IMI toxicity (Table 5.2.1, Fig. 5.2.2).

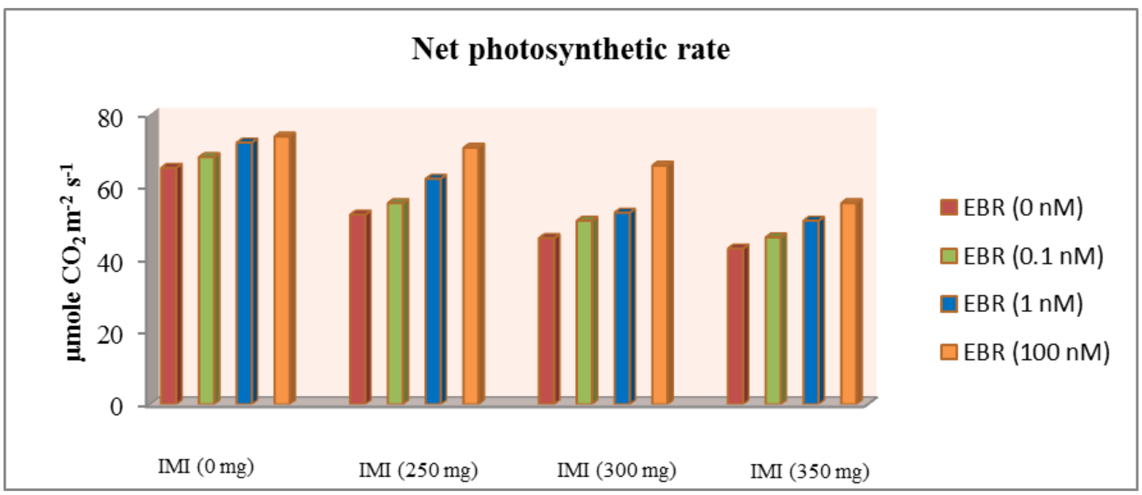

Fig. 5.2.2 Effect of seed soaking with EBR on net photosynthetic rate in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

Pn in 90 days old plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil) was maximum reduced to 29.8 µmole CO_2 m^{-2} s^{-1} in comparison to Pn of control plants (Pn = 47.9 µmole CO_2 m^{-2} s^{-1}). But, in plants raised from 100 nM EBR soaked seeds, the Pn was enhanced to 41.2 µmole CO_2 m^{-2} s^{-1} under IMI toxicity (Table 5.2.1, Fig. 5.2.3).

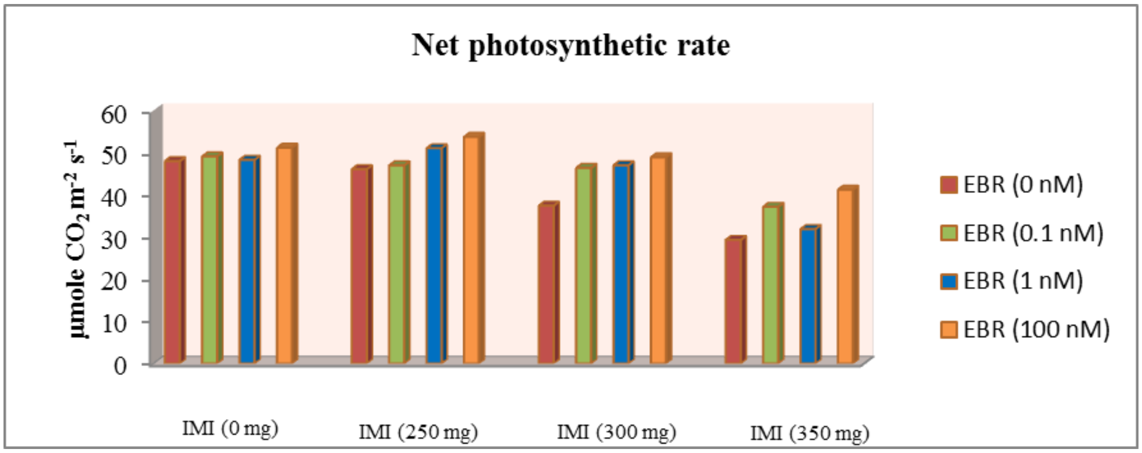

Fig. 5.2.3 Effect of seed soaking with EBR on net photosynthetic rate in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Data analysis using two-way ANOVA and Tukey's HSD showed significant differences for Pn in plants raised from EBR treated seeds and grown in IMI amended soils, as compared to the control plants (Table 5.2.1). MLR analysis also revealed that IMI concentration in soil decreased Pn (indicated by negative β_{IMI} values) whereas seed soaking with EBR increased the Pn in plants (indicated by positive β_{EBR} values) (Table 5.2.1). ANN model showed high correlation between target and output net photosynthetic rate values (Fig. 5.2.4)

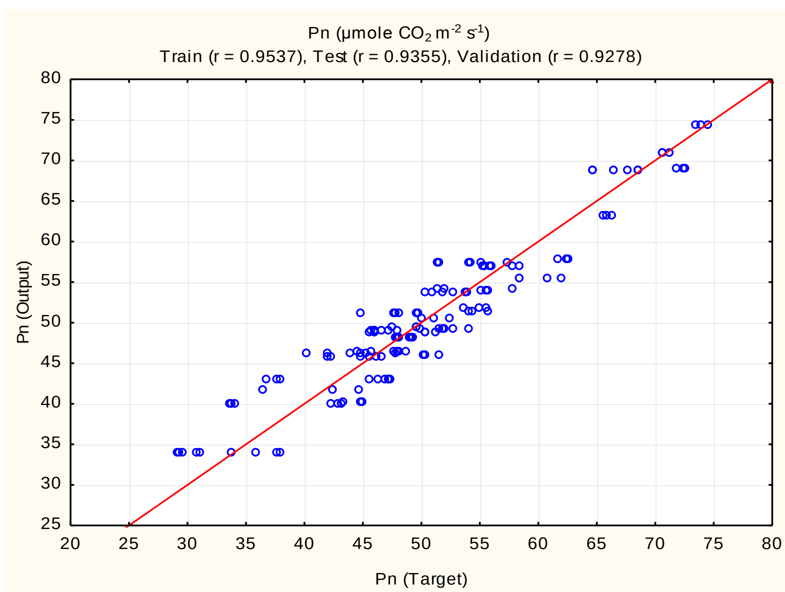

Fig. 5.2.4 Correlation between target (experimental) and output (simulated) net photosynthetic rates using ANN model (p<0.001).

Stomatal conductance (Gs)

It has been observed that Gs was declined with the increasing concentration of IMI in soils. In the leaves of 30 days old control plants, Gs was 2.1 mmole H_2O m^{-2} s^{-1} which was reduced to 1.5 mmole H_2O m^{-2} s^{-1} in the plants grown in soils mixed with 350 mg IMI Kg^{-1} soil. Moreover, in plants raised from EBR (100 nM) treated seeds and grown under IMI toxicity (350 mg IMI Kg^{-1} soil), Gs was further enhanced to 2.6 mmole H_2O m^{-2} s^{-1} (Table 5.2.2, Fig. 5.2.5).

Table 5.2.2 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on stomatal conductance 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		**Stomatal conductance (Gs) (mmole H_2O m^{-2} s^{-1})**		
IMI (mg Kg^{-1})	**EBR (nM L^{-1})**	**30 DAS**	**60 DAS**	**90 DAS**
0	0	2.1 ± 0.3	4.4 ± 0.3	2.3 ± 0.1
0	0.1	3.2 ± 0.3	5.8 ± 0.4	2.9 ± 0.1
0	1	2.4 ± 0.1	4.7 ± 0.3	3.1 ± 0.1
0	100	3.4 ± 1.8	6.1 ± 0.4	3.2 ± 0.3
250	0	2.2 ± 0.4	2.0 ± 0.2	1.8 ± 0.1
250	0.1	3.4 ± 0.5	2.84 ± 0.2	2.3 ± 0.7
250	1	3.4 ± 0.6	5.6 ± 0.9	2.4 ± 1.1
250	100	4.1 ± 0.6	7.3 ± 0.7	4.2 ± 1.1
300	0	1.5 ± 0.3	1.9 ± 0.1	1.5 ± 0.3
300	0.1	2.3 ± 0.6	2.9 ± 0.4	2.2 ± 0.3
300	1	2.4 ± 0.5	2.1 ± 0.1	2.1 ± 0.9
300	100	3.8 ± 0.2	4.6 ± 0.4	3.1 ± 0.2
350	0	1.5 ± 0.1	1.8 ± 0.2	0.8 ± 0.1
350	0.1	1.9 ± 3	1.9 ± 0.2	1.5 ± 0.3
350	1	2.5 ± 0.3	2.1 ± 0.2	1.6 ± 0.2
350	100	2.6 ± 0.1	2.4 ± 0.1	2.2 ± 0.1
Two way ANOVA				
F_{IMI}		7.3***	141.3***	16.0***
F_{EBR}		15.5***	80.7***	17.3***
$F_{IMI \times EBR}$		0.9	21.5***	1.2
HSD		1.8	1.2	1.5

Multiple linear regression			
MLR equation	**β-regression coefficients**		**r**
	β_{IMI}	**β_{EBR}**	
Gs (30 DAS) = 2.67 - 0.0011 IMI + 0.0108 EBR	- 0.1956	0.6139	0.7847***
Gs (60 DAS) = 5.05 - 0.0082 IMI + 0.0197 EBR	- 0.6219	0.4785	0.7847***
Gs (90 DAS) = 2.77 - 0.0031 IMI + 0.0109 EBR	- 0.5264	0.5918	0.7921***

*** indicates significant at p<0.001. r = multiple correlation coefficient. DAS = days after sowing.

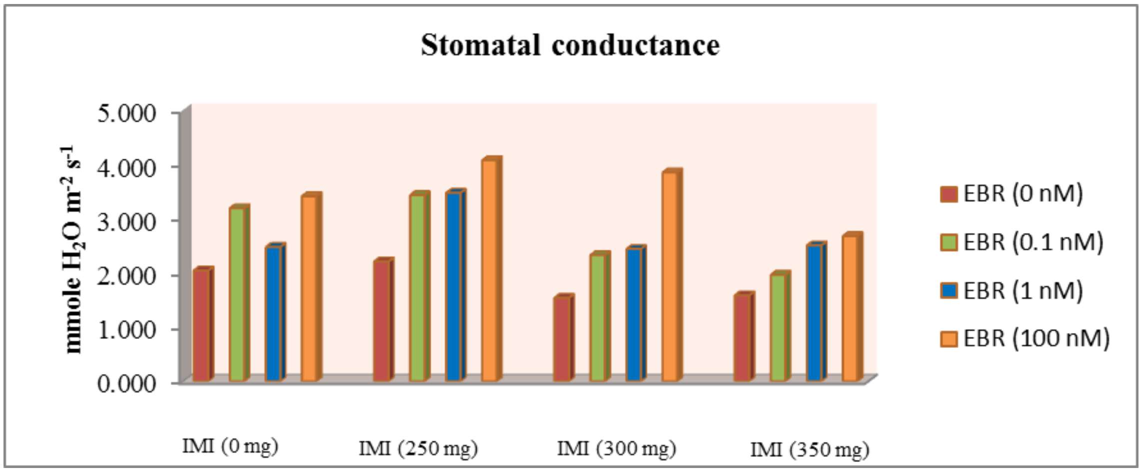

Fig. 5.2.5 Effect of seed soaking with EBR on stomatal conductance in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

Gs in 60 days old plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil) was reduced to 1.8 mmole H_2O m^{-2} s^{-1} as compared to Gs of 4.4 mmole H_2O m^{-2} s^{-1} in the control plants. Furthermore, EBR (100 nM) seed soaking resulted in recovery of Gs under IMI toxicity, which was enhanced to 2.4 mmole H_2O m^{-2} s^{-1} (Table 5.2.2, Fig. 5.2.6).

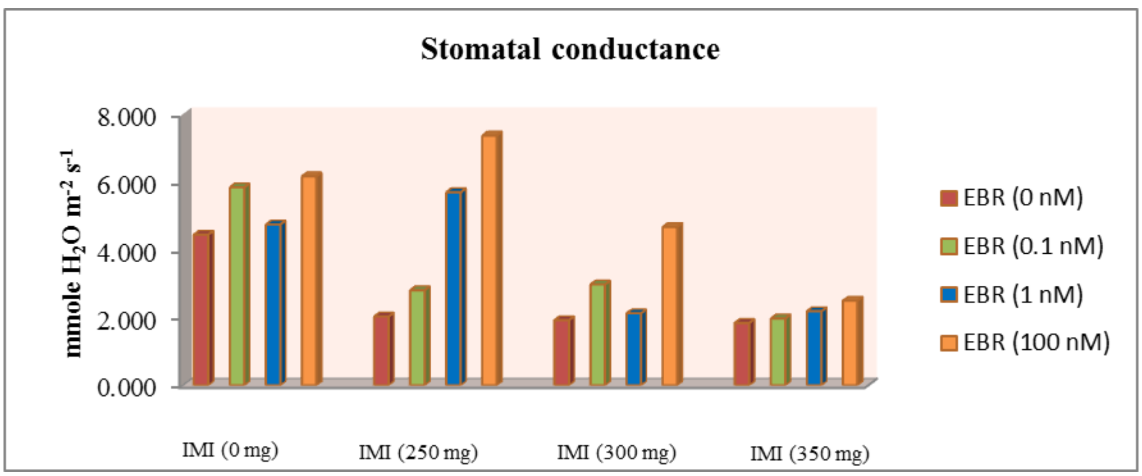

Fig. 5.2.6 Effect of seed soaking with EBR on stomatal conductance in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

Maximum decline in Gs was 0.8 mmole H_2O m^{-2} s^{-1} under IMI toxicity (350 mg IMI Kg^{-1} soil) when compared to Gs of 90 days old control plants (Gs = 2.3 mmole H_2O m^{-2} s^{-1}). Seed soaking with 100 nM EBR further enhanced the reduced Gs to 2.2 mmole H_2O m^{-2} s^{-1} under IMI toxicity (Table 5.2.2, Fig. 5.2.7).

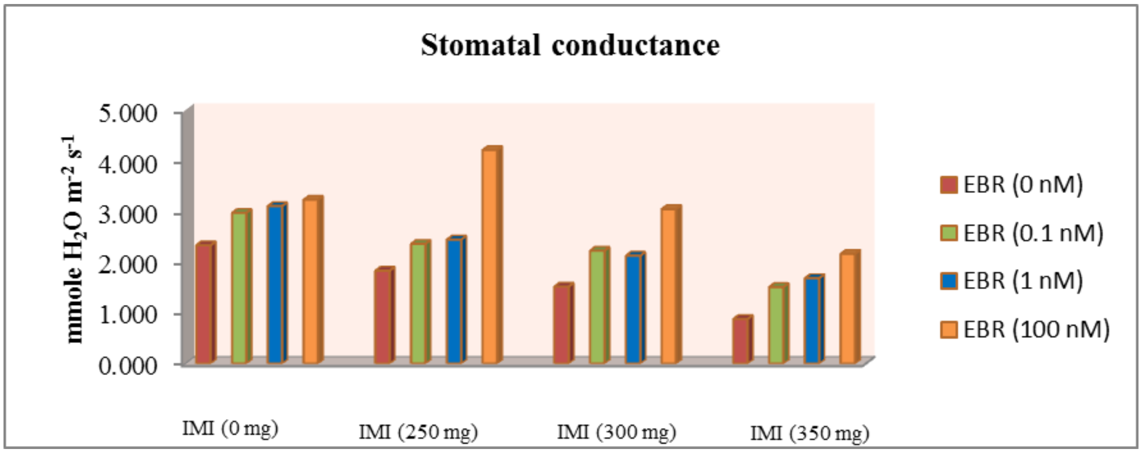

Fig. 5.2.7 Effect of seed soaking with EBR on stomatal conductance in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Two-way ANOVA and Tukey's HSD showed significant difference in Gs values for all the stages of plants. Data analysis using MLR revealed the role of IMI and EBR in Gs of leaves. IMI application reduced the Gs (shown by negative β_{IMI} values) whereas seed treatment with EBR enhanced the Gs in the leaves of *B. juncea* leaves as indicated by positive β_{EBR} values (Table 5.2.2). Data analysis using ANN revealed that ANN model simulated the experimental data at high level of significance ($p<0.001$) as shown in Fig. 5.2.8.

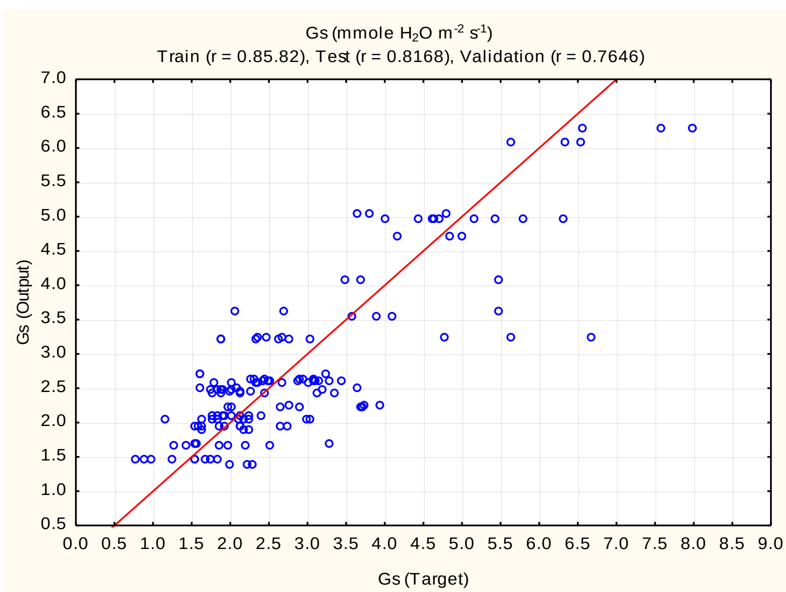

Fig. 5.2.8 Correlation between target (experimental) and output (simulated) stomatal conductance rates using ANN model (p<0.001).

Inter-cellular CO_2 (Ci)

In *B. juncea* plants grown in IMI amended soils, the Ci was reduced when compared to control plants. In 30 days old plants grown in IMI amended soils (350 mg IMI Kg^{-1} soil), Ci was slightly reduced to 239.5 µmole CO_2 $mole^{-1}$ as compared to Ci of control plants (246.8 µmole CO_2 $mole^{-1}$). However, in plants raised from EBR (100 nM) soaked seeds, Ci was increased to 250.8 µmole CO_2 $mole^{-1}$ under IMI stress (Table 5.2.3, Fig. 5.2.9).

Table 5.2.3 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on inter-cellular CO_2 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		Inter-cellular CO_2 (Ci) (µmole CO_2 $mole^{-1}$)		
IMI (mg Kg^{-1})	EBR (nM L^{-1})	30 DAS	60 DAS	90 DAS
0	0	246.8 ± 3.1	264.2 ± 1.3	242.0 ± 3.6
0	0.1	259.8 ± 6.6	267.2 ± 0.7	240.4 ± 2.1
0	1	259.1 ± 3.6	270.1 ± 1.6	245.7 ± 1.0
0	100	265.3 ± 0.7	270.7 ± 9.0	249.9 ± 0.3
250	0	245.8 ± 14.3	253.4 ± 9.9	230.4 ± 1.4
250	0.1	264.1 ± 9.5	259.0 ± 11.4	247.2 ± 0.9
250	1	269.4 ± 1.7	257.0 ± 15.5	261.3 ± 4.4
250	100	281.9 ± 0.9	282.2 ± 2.4	264.6 ± 3.8
300	0	248.7 ± 3.3	249.1 ± 29.9	227.3 ± 1.3
300	0.1	256.9 ± 13.3	260.5 ± 15.0	238.6 ± 2.4
300	1	259.4 ± 14.7	263.0 ± 2.5	252.3 ± 3.5
300	100	262.6 ± 7.8	271.4 ± 8.4	263.3 ± 1.9
350	0	239.5 ± 21.2	248.0 ± 5.5	218.9 ± 11.0
350	0.1	247.4 ± 15.4	256.6 ± 0.5	238.1 ± 7.9
350	1	245.2 ± 0.2	269.0 ± 5.2	245.2 ± 0.3
350	100	258.8 ± 1.1	274.6 ± 8.2	254.1 ± 1.9
Two way ANOVA				
F_{IMI}		6.5**	0.9	16.6***
F_{EBR}		10.1***	7.8***	109.0***
$F_{IMI \times EBR}$		0.6	0.8	7.3***
HSD		29.6	12.4	32.8

Multiple linear regression

MLR equation	β-regression coefficients		r
	β_{IMI}	β_{EBR}	
Ci (30 DAS) = 257.00 - 0.0156 IMI + 0.1275 EBR	- 0.1992	0.5620	0.5963*
Ci (60 DAS) = 264.10 - 0.0194 IMI + 0.1509 EBR	- 0.2851	0.7105	0.7656***
Ci (90 DAS) = 241.97 - 0.0062 IMI + 0.1759 EBR	- 0.0678	0.6094	0.6131*

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

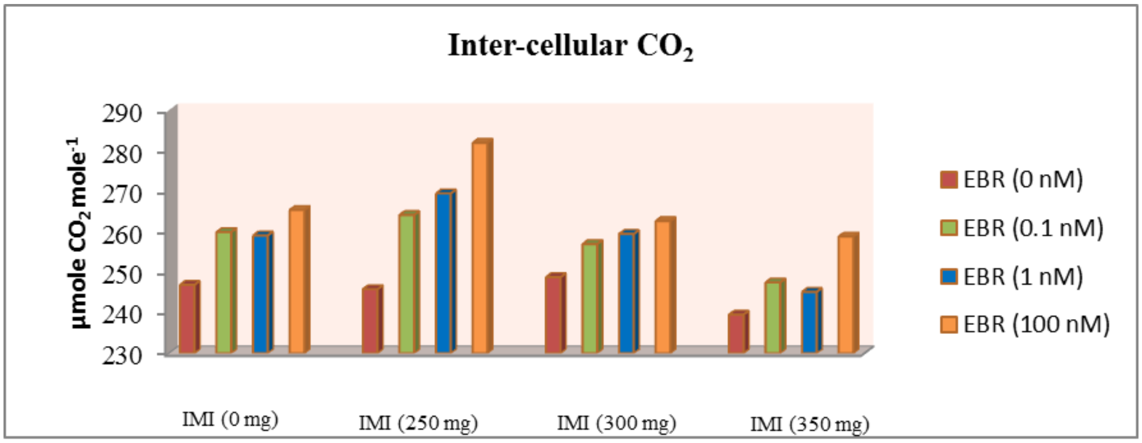

Fig. 5.2.9 Effect of seed soaking with EBR on inter-cellular CO_2 in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

In 60 days old plants, in comparison to control (Ci = 264.2 µmole CO_2 mole^{-1}), Ci was reduced to 248.0 µmole CO_2 mole^{-1} in plants grown in soils supplemented with 350 mg IMI Kg^{-1} soil. Seed soaking with EBR (100 nM) enhanced the Ci to 274.6 µmole CO_2 mole^{-1} in plants grown in soils with 350 mg IMI Kg^{-1} soil (Table 5.2.3, Fig. 5.2.10).

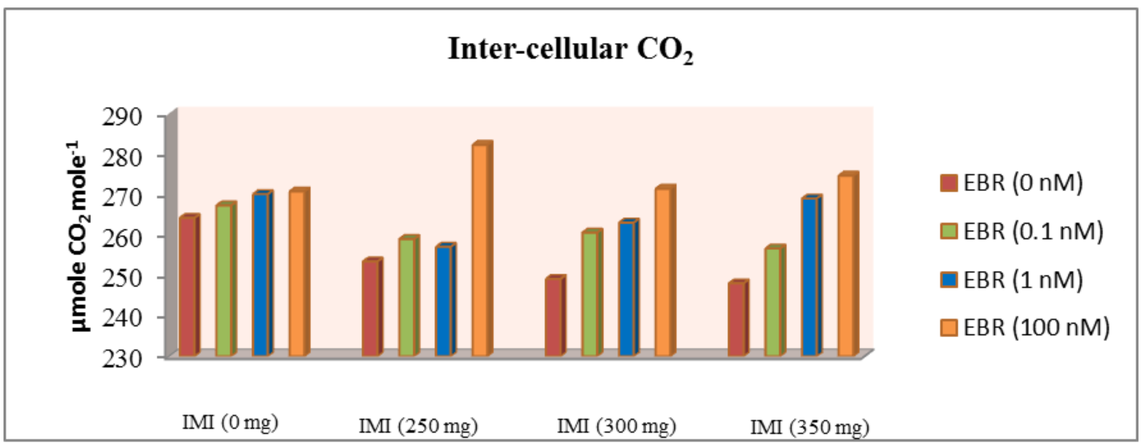

Fig. 5.2.10 Effect of seed soaking with EBR on inter-cellular CO_2 in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

Ci in 90 days old plants grown in IMI containing soils (350 mg IMI Kg^{-1} soil) was decreased to 218.9 µmole CO_2 mole^{-1} in comparison to Ci of control plants (Ci = 242.0 µmole CO_2 mole^{-1}). Increment in Ci (254.1 µmole CO_2 mole^{-1}) was observed in plants raised from EBR (100 nM) treated seeds and grown in soils amended with 350 mg IMI Kg^{-1} soil (Table 5.2.3, Fig. 5.2.11).

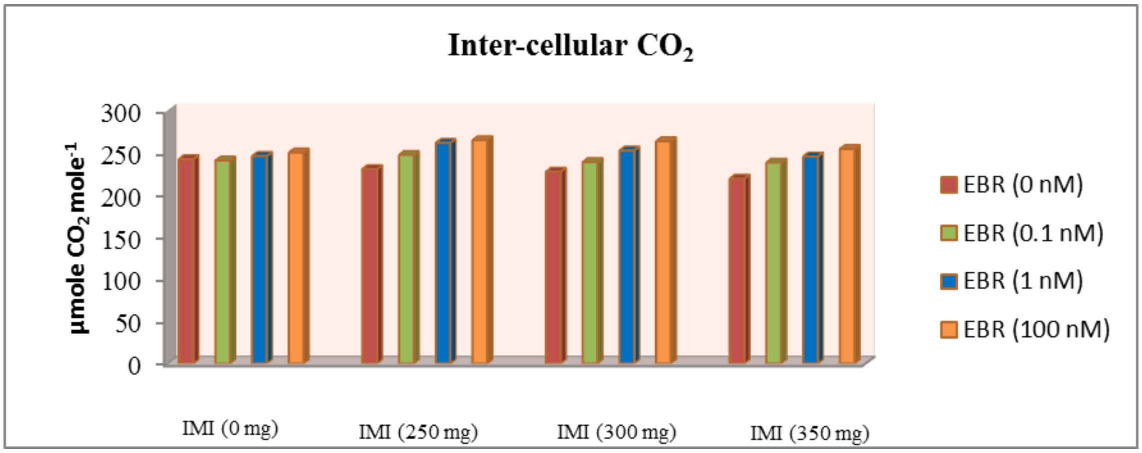

Fig. 5.2.11 Effect of seed soaking with EBR on inter-cellular CO_2 in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Analysis of data using two-way ANOVA and Tukey's HSD showed significant differences in Ci values indicating the effects of IMI and EBR on Ci. Furthermore, MLR analysis of data explained the effects of IMI and EBR on Ci. Negative β_{IMI} values indicated the reduction in Ci with IMI in soil, whereas positive β_{EBR} values suggested the increase in Ci in *B. juncea* plants raised from EBR treated seeds (Table 5.2.3). The target and output values from ANN for Ci were highly correlated as shown in Fig. 5.2.12.

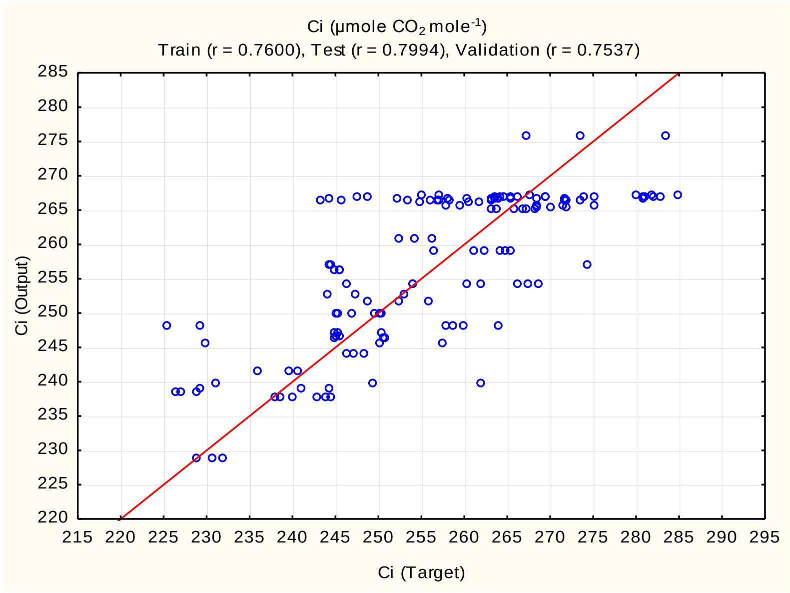

Fig. 5.2.12 Correlation between target (experimental) and output (simulated) inter-cellular CO_2 using ANN model (p<0.001).

Transpiration rate (Et)

In comparison to control plants, Et was observed to reduce in plants grown in IMI treated soils. As compared to Et of control plants (11.2 mmole H_2O m^{-2} s^{-1}), maximum reduction in Et (30 DAS) was 9.4 mmole H_2O m^{-2} s^{-1} in plants grown in soils mixed with 300 mg IMI Kg^{-1} soil. Seed soaking with 100 nM EBR resulted in increase of Et to 11.4 mmole H_2O m^{-2} s^{-1} in plants grown in soils amended with 300 mg IMI Kg^{-1} soil (Table 5.2.4, Fig. 5.2.13).

Table 5.3.4 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on transpiration rate 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		Transpiration rate (Et) (mmole H_2O m^{-2} s^{-1})		
IMI (mg Kg^{-1})	**EBR (nM L^{-1})**	**30 DAS**	**60 DAS**	**90 DAS**
0	0	11.2 ± 1.2	13.3 ± 0.1	10.7 ± 0.8
0	0.1	11.5 ± 0.1	13.5 ±0.1	11.4 ± 0.2
0	1	11.6 ± 0.1	13.1 ± 0.1	11.3 ± 0.1
0	100	12.4 ± 0.6	13.9 ± 0.3	12.1 ± 0.1
250	0	10.8 ± 0.3	10.8 ± 0.6	10.8 ± 0.2
250	0.1	11.5 ± 0.2	11.5 ± 0.3	10.8 ± 1.3
250	1	11.6 ± 0.4	13.5 ± 0.2	11.1 ± 1.4
250	100	13.1 ± 0.9	13.9 ± 0.3	12.4 ± 1.2
300	0	9.4 ± 0.4	10.4 ± 0.9	9.8 ± 0.3
300	0.1	10.5 ± 1.1	12.5 ± 0.4	11.4 ± 0.5
300	1	12.1 ± 0.5	10.9 ± 0.2	10.5 ± 0.5
300	100	11.4 ± 0.6	13.0 ± 0.2	10.2 ± 0.7
350	0	9.9 ± 0.1	10.9 ± 0.5	8.1 ± 0.5
350	0.1	10.0 ± 0.7	10.7 ± 0.3	9.9 ± 0.4
350	1	10.1 ± 0.1	10.9 ± 0.3	10.2 ± 0.7
350	100	11.3 ± 0.1	12.1 ± 0.3	10.8 ± 0.6
Two way ANOVA				
F_{IMI}		15.7***	69.3***	12.7***
F_{EBR}		17.4***	43.9***	8.6***
$F_{IMI \times EBR}$		2.2*	11.4***	2.1
HSD		1.8	1.2	2.2
Multiple linear regression				
MLR equation		**β-regression coefficients**		**r**
		$β_{IMI}$	**$β_{EBR}$**	
Et (30 DAS) = 11.61 - 0.0032 IMI + 0.0121 EBR		- 0.4539	0.5463	0.7103**
Et (60 DAS) = 13.23 - 0.0061 IMI + 0.0142 EBR		- 0.6568	0.4896	0.8193***
Et (90 DAS) = 11.38 - 0.0037 IMI + 0.0088 EBR		- 0.5185	0.3893	0.6485**

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

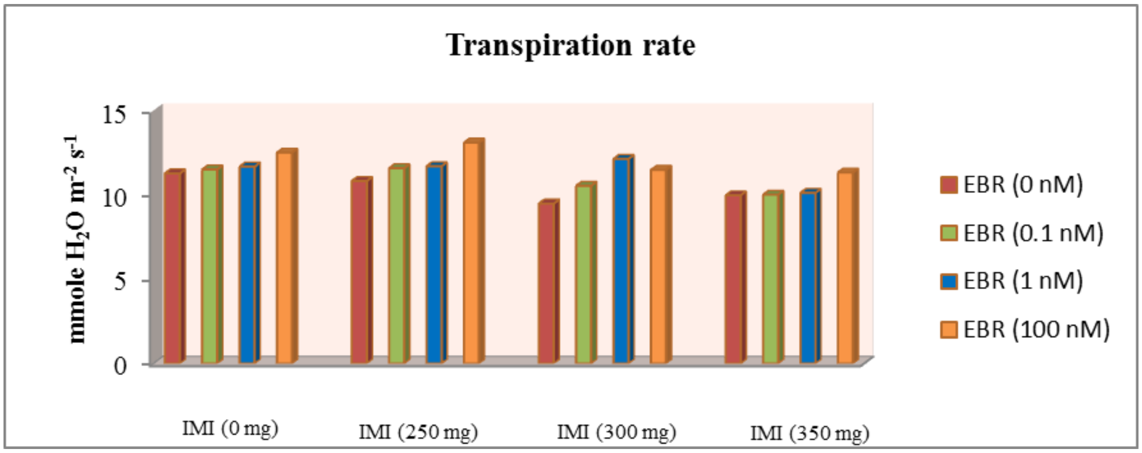

Fig. 5.2.13 Effect of seed soaking with EBR on transpiration rate in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.

When compared to the control (60 DAS), Et was maximum reduced to 10.4 mmole H_2O m^{-2} s^{-1} in plants grown in soils mixed with 300 mg IMI Kg^{-1} soil. Further enhancement in Et (13.0 mmole H_2O m^{-2} s^{-1}) was noticed in plants germinated from EBR (100 nM) soaked seeds and grown in pots containing 300 mg IMI Kg^{-1} soil (Table 5.2.4, Fig. 5.2.14).

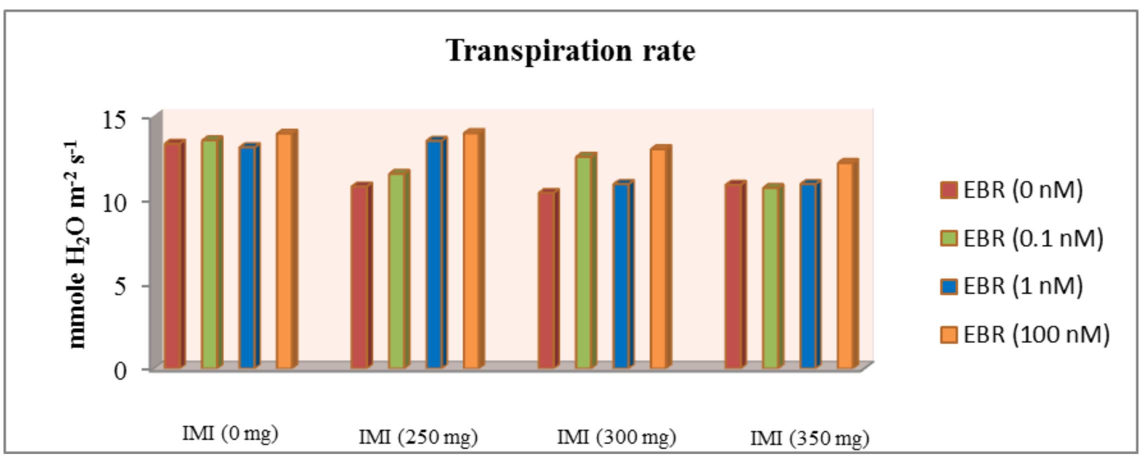

Fig. 5.2.14 Effect of seed soaking with EBR on transpiration rate in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.

As compared to the control (Et = 10.7 mmole H_2O m^{-2} s^{-1}) in 90 days old plants, Et was reduced to 8.1 mmole H_2O m^{-2} s^{-1} in plants grown under IMI toxicity (350 mg IMI Kg^{-1} soil). However, Et was observed to increase (10.8 mmole H_2O m^{-2} s^{-1}) in plants raised from EBR (100 nM) treated seeds and grown under IMI toxicity (Table 5.2.4, Fig. 5.2.15).

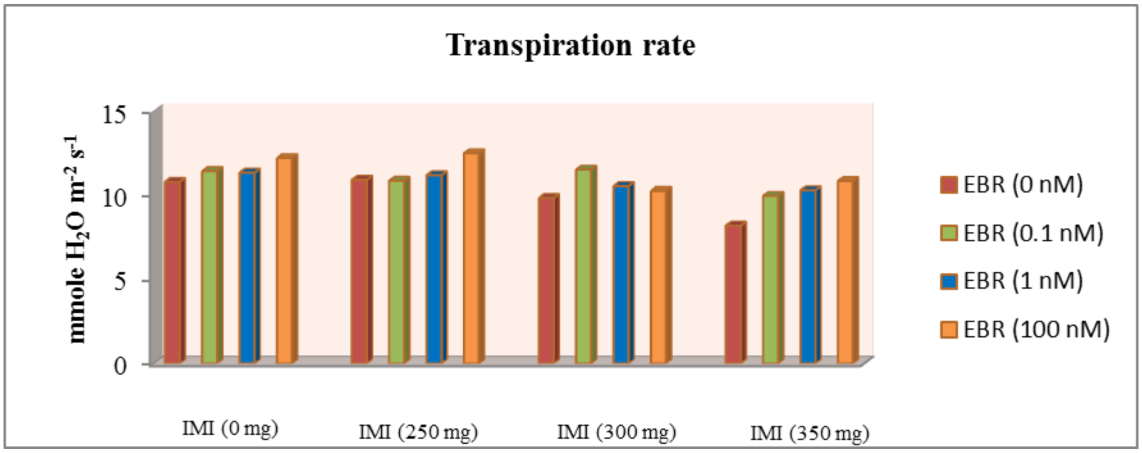

Fig. 5.2.15 Effect of seed soaking with EBR on transpiration rate in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.

Two-way ANOVA and Tukey's HSD showed significant differences in Et values of *B. juncea* plants. Furthermore, MLR analysis revealed that IMI application via soil reduced the Et (as indicated by negative β_{IMI} values) whereas seed treatment with EBR before sowing enhanced the Et in *B. juncea* plants under IMI toxicity which is indicated by positive β_{EBR} values (Table 5.2.4). Data analysis using ANN model showed that simulated and experimental values of Et were correlated after taking applied IMI, EBR and DAS as inputs (Fig. 5.2.16).

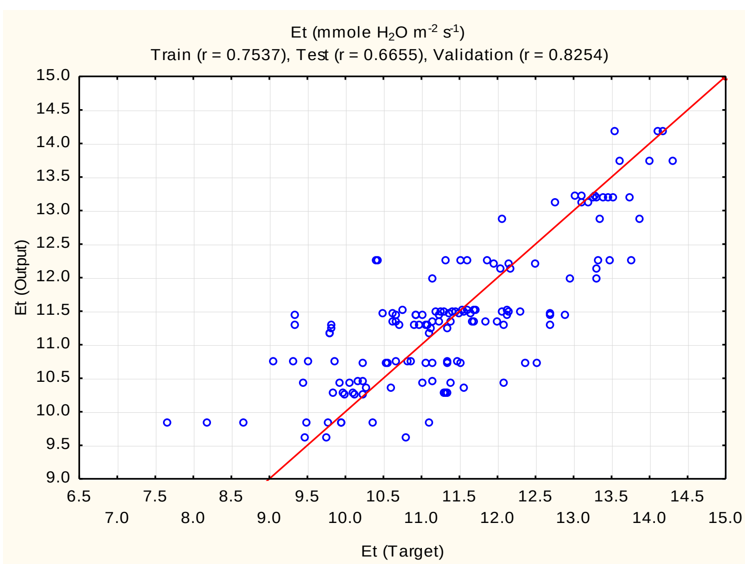

Fig. 5.2.16 Correlation between target (experimental) and output (simulated) transpiration rates using ANN model (p<0.001).