PHYSIOLOGICAL STUDIES ON IMPROVEMENT OF FABA BEAN Tolerance TO ABIOTIC STRESS BY Agrobacterium MEDIATED GENETIC TRANSFORMATION

By

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B.Sc. Agric. Sci. (Biotechniology), Fac. Agric., Cairo Univ., 2009

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ABSTRACT

Legume crops have been the primary targets for improvement by genetic transformation due to their importance for human and animal consumption worldwide. Many of these important legume crops were difficult to genetically engineer especially faba bean crop, mainly due to high phenolics content and their recalcitrance to in vitro regeneration. Therefore, a series of experiments were performed in order to evaluate the growth, morphological changes and production of phenolics in the in vitro plantlets of five Egyptian faba bean cultivars (Giza 843, Sakha 1, Sakha 3, Nubaria 2 and Nubaria 3). The obtained results revealed that cultivars Nubaria 2 and Sakha 3 had the highest regeneration frequency (85.3% and 78.6%), respectively, and low in total phenols concentration. The effect of different levels of chitosan (0, 2, 4, 8, 15, 30, 60, and 120 mg (1) on shoot regeneration from mature embryo axes of cv. Nubaria 2 were studied The obtained results indicated that high level of chitosan have lethal effect on the development of embryo axes. However, low levels of chitosan (2 mg/l and 4mg/l) in combination with 4.5 mg/l BAP had a positive effect on shoot regeneration. HPLC analysis showed changes in the polyphenols concentrations in faba bean plantlets that exposed to low levels of chitosan (2, 4 and 8 mg/l). The obtained results indicated also that the concentration of GA₃ and ABA were increased in the faba bean plantlets that exposed to the low levels of chitosan. The effect of salt stress imposed by the addition of sea salt at concentrations of 1000, 3000, 5000 and 7000 ppm to the culture media on the selected cultivars was studied .The results revealed that decreasing in plant growth sugars and phenols concentrations with increasing concentrations. This was highly pronounced in Sakha 3 cultivar followed by Nubaria2. the effect of antioxidants (DTT, Cystein and Sodium thiosulfate) on the transformation efficiency was studied. The results obtained indicated that the addition of DTT, cysteine and sodium thiosulfate to the culture media had positive effects on increasing the transformation efficiency of faba bean. Overexpression of PR10a gene from potato were studied in transgenic faba bean lines (T2 and T3) cv. Tattoo in comparison with wild type plants using physiological measurements (net photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll fluorescence Fv'/Fm' and electron transport rate ETR). This measurement were recorded using Li-Cor Li6400XT portable photosynthesis system (Licor ,Inc.,Lincoln, NE 68504) at 1200 PAR under drought and salt stress conditions using hydroponic culture system containing 150mM sodium chloride.

Key words: Embryo axes, regeneration frequency, phenols, GA₃, ABA, sugar, DTT, cysteine, sodium thiosulfate, PR10a, LI6400XT, PAR, net photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll fluorescence parameter Fv'/Fm', ETR.

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CONTENTS

REVIEW OF LITERATURE	
1. Legumes	
2. Faba bean (Vicia faba L.)	
2.1. Major constrains for faba bean productions	
3. Regeneration and genetic transformation in faba bean	
3.1. Effect of chitosan on enhancing shoot regeneration	
4. physiological response of plants to abiotic stresses (drought	t and
salt stresses)	
4.1. Salt and drought response related genes	
4.2.Dehydration Responsive Element Binding protein 2 A	
4.3. Pathogenesis - related protein 10a (PR10a) over expression h	ave
role in increasing plant tolerance to abiotic stress	
MATERIALS AND METHODS	
ESULTS AND DISCUSSION	
1. In vitro regeneration and transformation of faba bean	
1.1. Evaluation of regeneration capacity and phenolic compound	l for
five Egyptian faba beancultivars	
1.2. Effect of different chitosan levels on shoot regeneration	
1.3. Effect of chitosan on total soluble phenols	
1.4. Estimation of plant hormones (GA ₃ and ABA)	
1. Effect of salt stress on the concentrations of chlorophyll, sugars, phenols, activity of antioxidant enzymes and growth <i>vitro</i> plantlets of faba bean cultivars (Sakha 3 and Nubaria 2)	of i
2.1. Growth characters	
2. 2. Chlorophyll determination.	
2.3. Elements analysis	
2.4. Sugars determination	
2.5. Phenols determination	
2.6.Activity of antioxidant enzymes	
3. Establishment of a transformation protocol in faba bean	
cultivars (Sakha 3, Nubaria 2).	
3.1. Selection effect of the herbicide PPT on the regener	ration
potential of embryo axes of faba bean (<i>Vicia faba</i> L.)	

CONTENTS (Cont.)

experiments. 3.2.1. Assessment of factors affecting <i>Agrobacterium</i> -mediated transformation of faba bean. 4. Recovery of the transgenics by micrografting. 5. Analysis of the transgenics. 5.1. Herbicide (BASTA®) leaf paint test. 5.2. PCR analysis of the transgenics. 5.3. Evaluation of drought stresses tolerance of PR10a transgenic faba bean lines. 5.3.1. Phenotype of the transgenic faba bean plants under drought stress. 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g _s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines. 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines. 5.4.1 Effect of salt stress on faba bean growth. 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter. 5.4.3. Effects of salt stress on gas-exchange parameters: 5.4.4. Osmotic potential.
transformation of faba bean
 4. Recovery of the transgenics by micrografting 5. Analysis of the transgenics 5.1. Herbicide (BASTA®) leaf paint test 5.2. PCR analysis of the transgenics 5.3. Evaluation of drought stresses tolerance of PR10a transgenic faba bean lines 5.3.1. Phenotype of the transgenic faba bean plants under drought stress 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines 5.4.1 Effect of salt stress on faba bean growth 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter 5.4.3. Effects of salt stress on gas-exchange parameters:
 5. Analysis of the transgenics. 5.1. Herbicide (BASTA®) leaf paint test 5.2. PCR analysis of the transgenics. 5.3. Evaluation of drought stresses tolerance of PR10a transgenic faba bean lines. 5.3.1. Phenotype of the transgenic faba bean plants under drought stress. 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines. 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines. 5.4.1 Effect of salt stress on faba bean growth. 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter. 5.4.3. Effects of salt stress on gas-exchange parameters:
 5.1. Herbicide (BASTA®) leaf paint test 5.2. PCR analysis of the transgenics 5.3. Evaluation of drought stresses tolerance of PR10a transgenic faba bean lines 5.3.1. Phenotype of the transgenic faba bean plants under drought stress 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines 5.4.1 Effect of salt stress on faba bean growth 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter 5.4.3. Effects of salt stress on gas-exchange parameters:
 5.2. PCR analysis of the transgenics
 5.3. Evaluation of drought stresses tolerance of PR10a transgenic faba bean lines 5.3.1. Phenotype of the transgenic faba bean plants under drought stress 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines 5.4.1 Effect of salt stress on faba bean growth 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter 5.4.3. Effects of salt stress on gas-exchange parameters:
faba bean lines
 5.3.1. Phenotype of the transgenic faba bean plants under drought stress. 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines. 5.4. Evaluation of salt stress tolerance of PR10a transgenic faba bean lines. 5.4.1 Effect of salt stress on faba bean growth. 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter. 5.4.3. Effects of salt stress on gas-exchange parameters:
drought stress
 5.3.2. Analysis of photosynthetic rate (A), stomatal conductance (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines
 (g_s), transpiration- rate (E), cholorophyll fluorescence parameters in faba bean PR10a transgenic lines
 5.4.1 Effect of salt stress on faba bean growth 5.4.2. Chlorophyll content estimation by SPAD chlorophyll meter. 5.4.3. Effects of salt stress on gas-exchange parameters:
5.4.5.Performance of PR10a transgenic faba bean under salt stress. 5.4.6. Biomass. 5.4.7. Elements analysis.
CONCLUSIONSUMMARY
REFERENCESARABIC SUMMARY

LIST OF TABLES

No	Title	Page
1.	Composition of Sea Salts (Sigma Aldrich; Product number S9883)	26
2.	Composition of the culture media used in this study	27
3.	MS and B5 Media composition	28
4.	Shoot regeneration percentage from embryonic axes from five faba bean cultivars	60
5.	Shoot height, shoots number per explants, node number per shoot, fresh weight and dry weight of faba bean after 8 weeks of culturing the embryo axes explants on SIM (Shoot Induction Medium)	61
6.	Determination of total, free and conjugated phenolic compounds in the regenerated plantlets of five faba bean cultivars	63
7.	Effect of different concentrations of chitosan on the regeneration potential of embryo axes of faba bean cv. Nubaria2, Fresh weight (FW), dry weight (DW), shoot length, shoots number per explant, leaves number after 6 weeks on SIM (Shoot Induction Medium) containing different chitosan level (0, 2, 4 and 8mg chitosan /1)	65
8.	Determination of total soluble phenols $(\mu g/g)$ in the regenerated faba bean cv. Nubaria 2 ,shoots on SIM medium (Shoot Induction Medium) containing different levels of chitosan	69
9.	Determination of phenolic concentrations in regenerated faba bean shoots on SIM medium (Shoot Induction Medium) containing different levels of chitosan using HPLC	70
10.	Determination of plant hormones (GA3 and ABA) concentration in regenerated faba shoots on SIM medium (Shoot Induction Medium) containing different concentrations of chitosan using HPLC after culturing the embryo axes for 6 weeks	75