Deciphering *In Vitro* Regeneration Capabilities of Wheat and Investigations of Genetic Transformation in Wheat and Barley

By

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I, Ummara Waheed, CIIT/FA09-R62-001/ATD hereby declare that I have produced the work presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except referred to wherever due, and that the amount of plagiarism is within acceptable range. If a violation of rules on research has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of the HEC.

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It is certified that Miss Ummara Waheed, CIIT/FA09-R62-001/ATD has carried out all the work related to this thesis under my supervision in Biotechnology at the Department of Environmental Sciences, COMSATS Institute of Informational Technology, Abbottabad and the work fulfills the requirements for award of PhD degree.

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DEDICATION

To

My parents
(Abu and Jiya)

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ABSTRACT

Deciphering *In Vitro* Regeneration Capabilities of Wheat and Investigations of Genetic Transformation in Wheat and Barley

An efficient and reliable genetic transformation system is imperative for the improvement of food grains such as wheat and barley. While wheat transformation is complex due to its larger genome and high ploidy level, the barley has a limiting factor of genotypic dependency. In addition, cereals are known to be recalcitrant towards callus induction and regeneration. The biological processes behind *in vitro* response are complex and poorly understood. Selection of responsive genotypes and suitable media for tissue culture are important for genetic transformation. Mature embryos of wheat cultivars, lines and special stocks were used to evaluate genotypic and chromosomal response to tissue culture with variable concentrations of 2, 4-D in MS-medium. Similarly, different concentrations of IAA, BAP and Kinetin were used to find optimum combinations for regeneration. Specific expression vector pBRACT 214-*NDPK2* carrying *NDPK2* gene was used to compare relative *Agrobacterium* mediated transformation efficiency in wheat and barley. Significant differences were found among mean values of calli obtained under different concentrations of 2, 4-D for the tested wheat cultivars and lines. Callus induction frequency varied widely with genotype and exogenous auxin source ranging from 21% (Chenab 2000) to 94% (Atta Habib) at 1 and 2 mg/L of 2, 4-D, respectively. Most responsive cultivars and lines were Atta Habib, Siran, Iqbal 2000, Inqalab 2000, Marvi 2000, CIITADSW2, CIITADSW4, CIITADSW5 and CIITADSW9 which yielded maximum calli in a minimum time period of four weeks. It was found that from genome A the chromosomes 1A, 2A showed marked effect on callus induction, while from B and D genome the chromosome 3B, 7B, 2D, 4D and 6D were found responsible for the callus induction response. Based on the information from the response of substitution lines, the gene responsible for tissue culture response can be marked on to the individual chromosomes. Most efficient regeneration response was shown in Atta Habib followed by Siran and Chenab 2000 respectively. Wheat line CIITADSW5 showed significantly highest regeneration potential of 31% followed by CIITADSW1, CIITADSW4, CIITADSW5 and CIITADSW9 each with 25%. Both wheat and barley showed different responses towards callus induction and regeneration. Both embryogenic and non embryogenic calli were found in wheat with significantly greater tendency for embryogenecity in barley. The barley transformed lines showed good response on the regeneration medium as compared to wheat. PCR analysis of putative transformants using genomic DNA analyses showed a promising transformation response in barley with 27% transformation efficiency opposed to wheat where no true transgenic was obtained in any cultivar used in this study. The protocol developed and optimized for wheat and barley transformation will greatly help in crop improvement programme through genetic engineering especially in diploid relatives of cereals. Findings of this study suggested that callus induction and regeneration were genotype and hormones dependent, but independent of each other.

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LIST OF ABBREVIATIONS

mMT	Million metric tons
DNA	Deoxyribo nucleic acid
2,4-D	2, 4-Dichlorophenoxyacetic acid
IBA	Indole Butyric Acid
MS	Murashige and Skoog
LB	Luria-Bertani
BAP	6-benzylaminopurine
IBA	Indole Butyric Acid
IAA	Indole Acetic Acid
BAP	Benzyl Amino Purine
M	Molar
uM	Micro Molar
mL	Milli liter
mg	Milli gram
mm	Milli meter
ANOVA	Analysis of variance
SPSS	Statistical package for the social sciences
NDPK2	Nucleoside diphosphate kinase
MPK	Mitogen activated protein kinases
CAT	Catalase
Phy A	Phytochrome A
CDS	Coding DNA sequence
E. coli	*Escherichia coli*
BRACT	Biotechnology Resources for Arable Crop Transformation
JIC	John Innes Centre
Cv.	Cultivar
PCR	Polymerase Chain Reaction