

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



By

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CIIT/FA09-R62-001/ATD

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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 embryogenicity 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.

TABLE OF CONTENTS

1	Introduction.....	1
1.1	Wheat origin evolution and classification.....	2
1.2	Wheat cultivation	4
1.2.1	Major cultivated species of wheat.....	5
1.2.1.1	Hexaploid wheat species.....	6
1.2.1.2	Tetraploid wheat species.....	6
1.2.1.3	Diploid wheat species.....	6
1.2.2	Types of wheat and their utilization.....	6
1.2.2.1	Hard red spring.....	6
1.2.2.2	Hard red winter.....	7
1.2.2.3	Soft red winter.....	7
1.2.2.4	Hard white.....	7
1.2.2.5	Soft white.....	7
1.3	Wheat production	7
1.4	Wheat nutrition.....	9
1.4.1	Cytogenetics of wheat	9
1.5	History of plant tissue culturing.....	10
1.5.1	Wheat tissue culturing.....	11
1.5.2	Factors effecting <i>in vitro</i> culture of wheat.....	13
1.5.2.1	Explants.....	13
1.5.2.2	Effect of Media on wheat tissue culture.....	16
1.5.2.3	Genotype effect.....	19
1.6	Wheat transformation.....	22
1.6.1	Different methods of wheat transformation.....	22
1.6.1.1	Protoplasts transformation.....	23
1.6.1.2	Transformation through electroporation.....	24
1.6.1.3	Transformation through particle projectile bombardment.....	24

1.6.1.4	<i>Agrobacterium</i> -mediated transformation.....	25
1.7	Barley.....	27
1.7.1	Origin and classification of barley.....	27
1.7.1.1	Cytogenetic of barley.....	27
1.7.1.2	Barley cultivation.....	27
1.7.1.3	Barley production.....	28
1.7.1.4	Barley consumption.....	29
1.7.2	Barley transformation.....	29
1.7.2.1	Different methods employed for barley transformation.....	29
1.7.2.2	Biolistic transformation in barley.....	30
1.7.2.3	<i>Agrobacterium</i> -mediated barley transformation	30
1.8	Objectives	33
2	Materials and Methods.....	34
2.1	Screening of wheat cultivars and lines for callus induction	35
2.1.1	Plant material	35
2.1.2	Chemicals.....	36
2.1.3	Stock solutions preparations.....	36
2.1.4	Callus induction medium.....	36
2.1.5	Sterilization and explants preparation for callus induction..	36
2.1.6	Isolation of mature embryos from seeds	37
2.1.7	Callus induction.....	37
2.1.8	Parameters for callus induction.....	38
2.1.8.1	Days to callus induction.....	38
2.1.8.2	Effect of of 2, 4-D on callus induction.....	38
2.1.8.3	Measurement of callus growth.....	38
2.1.8.4	Callus morphology.....	38
2.1.8.5	Genotypic response to callus induction.....	38
2.2	Callus induction in chromosomal substitution lines	39

2.2.1	Chromosomal substitution lines	39
2.2.2	Callus induction.....	40
2.3	Wheat Regeneration.....	41
2.3.1	Regeneration medium.....	41
2.3.2	Stock solutions used in regeneration.....	41
2.3.2.1	Indole acetic acid (IAA)	41
2.3.2.2	Kinetin.....	41
2.3.2.3	Benzyl amino purine (BAP).....	41
2.3.3	Regeneration.....	41
2.3.4	Statistical analysis.....	42
2.4	Construction of plant expression vector for wheat and barley transformation.....	43
2.4.1	Materials used during construction of plant expression vector	43
2.4.2	Equipment used during construction of plant expression vector.....	44
2.4.3	Different stages involved during gateway cloning.....	44
2.4.3.1	PCR amplification of <i>NDPK2</i> gene.....	46
2.4.3.2	Cloning of <i>NDPK2</i> gene into entry vector.....	46
2.4.3.3	Cloning of <i>NDPK2</i> gene into destination vector pBRACT-214.....	48
2.4.4	Transformation of pBRACT 214- <i>NDPK2</i> vector into <i>Agrobacterium tumefaciens</i> strain AGL1.....	50
2.5	Genetic transformation of barley and wheat with pBRACT 214- <i>NDPK2</i>	51
2.5.1	Wheat transformation.....	51
2.5.1.1	Plant material used for wheat transformation.....	51
2.5.1.2	Chemicals.....	52
2.5.1.3	Plant tissue culture media and stock solutions....	52
2.5.1.4	Stock solutions.....	53
2.5.1.5	Plant expression vector.....	55
2.5.1.6	Bacterial culture medium.....	56

2.5.1.7	Preparation of <i>Agrobacterium</i> standard inoculum.....	57
2.5.1.8	Growth of <i>Agrobacterium tumefaciens</i> strain AGL1.....	57
2.5.1.9	Preparation of tissue culture media.....	57
2.5.1.10	Growth of plant material.....	58
2.5.1.11	<i>Agrobacterium</i> -mediated transformation of wheat.....	59
2.5.2	Transformation studies in barley.....	61
2.5.2.1	Plant material.....	61
2.5.2.2	Media and stock solutions.....	62
2.5.2.3	Stocks solutions preparation.....	63
2.5.2.4	Media preparation.....	64
2.5.2.5	Preparation of <i>Agrobacterium</i> standard inoculum.....	64
2.5.2.6	Growth of <i>Agrobacterium tumefaciens</i> strain AGL1.....	65
2.5.2.7	Collection and sterilization of immature embryos.....	65
2.5.2.8	Isolation of immature embryos and embryonic axis removal.....	65
2.5.2.9	<i>Agrobacterium</i> inoculation and co-cultivation....	66
2.5.2.10	Selection.....	66
2.5.2.11	Transition stage.....	67
2.5.2.12	Regeneration of transgenic plants.....	67
2.5.2.13	Plantlet transfer to soil.....	67
2.5.2.14	PCR based screening of putative transgenic lines	67
3	Results.....	69
3.1	Callus induction in wheat cultivars.....	70
3.1.1	Days to callus induction in wheat cultivars.....	70
3.1.2	Morphology of produced callus.....	71
3.1.3	Total callus production	73
3.1.4	Percent callus induction in wheat cultivars at varying concentrations of 2,4-D	74

3.1.5	Genotypic response of wheat cultivars towards callus induction.....	78
3.2	Callogenesis performance of advanced wheat lines	78
3.2.1	Days to callus induction in wheat lines	78
3.2.2	Total callus production in wheat lines.....	79
3.2.3	Percent callus induction in wheat lines at varying 2,4-D concentrations.....	80
3.2.4	Analysis of genotypic response of wheat lines towards callus induction.....	82
3.3	Callus induction responses in chromosome substitution lines.....	83
3.3.1	Callus induction response of genome A substituted Cheyenne and Wichita lines.....	84
3.3.2	Callus induction response of genome B substituted lines in Cheyenne and Wichita lines.....	86
3.3.3	Callus induction response of genome D substituted lines...	87
3.4	Regeneration in wheat cultivars.....	88
3.4.1	Days to regeneration in wheat cultivars.....	88
3.4.2	Regeneration response of wheat cultivars at varying concentrations of IAA and Kinetin	90
3.4.3	Genotypic response of wheat cultivars towards regeneration.....	92
3.5	Regeneration in wheat lines.....	94
3.5.1	Days to regeneration in wheat lines.....	94
3.5.2	Regeneration percentage in wheat lines.....	96
3.5.3	Regeneration potential in wheat lines.....	100
3.6	Construction of plant expression vector for <i>Agrobacterium</i> -mediated transformation of wheat and barley.....	102
3.6.1	<i>NDPK2</i> gene amplification.....	102
3.6.2	Cloning of <i>NDPK2</i> into Entry Clone.....	103
3.6.3	Destination Vector.....	104
3.7	Wheat and barley transformation.....	106
3.7.1	<i>Agrobacterium</i> -mediated wheat transformation.....	106
3.7.2	<i>Agrobacterium</i> -mediated barley transformation.....	112

4	Discussion.....	117
4.1	Callus induction and regeneration.....	118
4.1.1	Callus induction.....	118
4.1.2	Regeneration.....	121
4.1.3	Chromosome identification of callus induction.....	124
4.2	<i>Agrobacterium</i> -mediated transformation of wheat and barley.....	125
4.2.1	Genetic transformation of wheat.....	125
4.2.2	Genetic transformation of barley	129
4.3	Conclusions.....	132
4.4	Recommendations.....	133
5	References	134

LIST OF FIGURES

Fig. 1.1	Wheat evolution and polyploidization in the history.....	4
Fig. 2.1	Sterilized mature embryos.....	37
Fig. 2.2	Imbibed embryo after 20 hours.....	37
Fig. 2.3	Embryos arranged on callus induction medium.....	37
Fig. 2.4	Schematic presentation of parent pCAMBIA vector.....	43
Fig. 2.5	Strategy for the construction of plant expression vector through gateway cloning	45
Fig. 2.6	Schematic diagram of pBRACT 214 vector.....	49
Fig. 2.7	Electroporation machine.....	51
Fig. 2.8	Schematic representation of pBRACT 214- <i>NDPK2</i> vector.....	56
Fig. 2.9	Wheat spike collected after 14 days anthesis.....	59
Fig. 2.10	Embryonic axis removal from the embryo using the fine forceps...	60
Fig. 2.11	Embryo showing suitable size.....	65
Fig. 2.12	Sterilized barley seeds.....	65
Fig. 2.13	Immature embryos after removal of embryonic axis.....	66
Fig. 3.1	Appearance of white translucent tissue at the callus initiation from mature embryo.....	70
Fig. 3.2	Callus induction initiation in wheat cultivars against different concentrations of 2, 4-D hormone. Vertical bars indicate calli induction frequency at each concentration. Different alphabets on the bars represent significant difference in the callus induction.	71
Fig. 3.3	Change in callus texture and color (a) Explants after one week on callus induction medium with less compact structure (b) Formation of nodules on callus surface after three weeks.....	72
Fig. 3.4	Callus morphology (a) Embryogenic callus formation with compact nodules (b) Non embryogenic calli showing watery appearance.....	73
Fig. 3.5	Weight of callus of wheat cultivars after four weeks at varying	

	concentrations of 2,4-D. Vertical bars indicate calli induction frequency at each concentration. Different alphabets on the bars represent significant difference in the callus induction.....	74
Fig. 3.6	Comparison of callus induction frequency at different concentration of 2,4-D ranging from 1-5 mg/L. The vertical bars indicate the calli induction frequency at each concentration. Different alphabets on the bars represent the significant difference in the callus induction of wheat cultivars at 5 different 2,4-D concentration.....	75
Fig. 3.7	Cumulative callus induction pattern of varying concentrations of 2,4-D tested against wheat cultivars. Vertical bars represent the callus induction potential of each tested 2,4-D concentration used for wheat cultivars. Bars with asterisk signs represent significant differences among different concentrations.....	76
Fig. 3.8	Cumulative callus induction pattern of all the tested wheat cultivars against varying 2,4-D concentrations. The vertical bars indicate the callus frequency; bars with asterisk represent the cultivars producing significantly higher callus induction.....	78
Fig. 3.9	Callus induction initiation in wheat lines 2 and 3 mg/L concentrations of 2,4-D.....	79
Fig. 3.10	Wheat cultivars showing weight (mg) after four weeks at varying 2,4-D concentrations.....	80
Fig. 3.11	Comparison of callus induction frequency at 2 and 3 mg/L concentration of 2,4-D. Vertical bars represent frequency of calli induction at each concentration. Different alphabets on the bars indicate significant difference in callus induction of wheat lines at tested concentrations of 2,4-D.....	81
Fig. 3.12	Cumulative callus induction pattern of 2 and 3 mg/L of 2,4-D tested against wheat lines. Vertical bars represent the callus induction potential of each tested 2,4-D concentration used for wheat cultivars. Bars with asterisk signs represent significant differences between both concentrations.....	81
Fig. 3.13	Cumulative callus induction pattern of all the tested wheat lines against varying 2,4-D concentrations. The vertical bars indicate the callus frequency; bars with asterisk represent the lines producing significantly higher callus induction.....	82

Fig. 3.14	Comparison of callus induction frequency of genome A substituted lines at 2 mg/L 2,4-D. The bars indicate the calli induction frequency. Different alphabets on the bars represent the significant difference in the callus induction.....	85
Fig. 3.15	(a) Positive callus induction response of substituted 1A chromosome of WI (CNN) is shown on the half panel of petri plate while negative callus induction is shown in its corresponding population on the same petri plate (b) Positive callus induction response of substituted 2A chromosome of CNN (WI) on the half panel of petri plate while negative callus induction is shown in its corresponding population on same plate.	85
Fig. 3.16	Comparison of callus induction frequency of genome B substituted lines at 2 mg/L 2,4-D. The bars indicate the calli induction frequency. Different alphabets on the bars represent the significant difference in the callus induction.....	86
Fig. 3.17	Positive callus induction CNN (WI) 3B response in 3B chromosomes of CNN (WI) while negative callus induction is shown in its corresponding population of WI (CNN) 3B (b) Positive callus induction WI (CNN) 7B response in 7B chromosomes of CNN (WI) while negative callus induction is shown in its corresponding population of WI (CNN) 3B.....	87
Fig. 3.18	Comparison of callus induction frequency of genome D substituted lines recorded. The bars indicate the calli induction frequency. The vertical lines on each bar are the error bars showing the deviation from the mean within each replication.....	87
Fig. 3.19	Positive callus induction response in 4D chromosomes of CNN (WI) while negative callus induction is shown in its corresponding line (b) positive callus induction WI(CNN6D) while negative callus induction is shown in its corresponding line.....	88
Fig. 3.20	Representative figure showing the green spots on the callus surface as early signs of regeneration.....	89
Fig. 3.21	Regeneration initiation in wheat cultivars against different combinations of IAA and Kinetin.....	90
Fig. 3.22	Comparison of regeneration frequency at different concentration of IAA and Kinetin. The vertical bars indicate the regeneration frequency at each concentration. The vertical lines on each bar are the error bars showing the deviation from the mean within each replication. A different alphabet on the bars represents the	

	significant difference within varying IAA and kinetin concentrations for each wheat cultivar.....	91
Fig. 3.23	Cumulative regeneration pattern obtained at selected hormonal concentrations (IAA and Kinetin) against wheat cultivars. The vertical bars indicate the regeneration frequency. Bars with asterisk signs show significant differences among the other concentrations.....	92
Fig. 3.24	Cumulative regeneration pattern in wheat cultivars against varying concentrations of IAA and Kinetin. Bars with asterisk signs show significant differences among wheat cultivars.....	94
Fig. 3.25	Regeneration initiation in wheat lines (a) against different combinations of IAA and Kinetin (b) against different combinations of IAA and BAP.....	96
Fig. 3.26	Comparison of regeneration frequency at different concentration of (a) IAA and BAP (b) IAA and Kinetin. The bars indicate the regeneration frequency at each concentration. The vertical lines on each bar are the error bars showing the deviation from the mean within each replication.....	98
Fig. 3.27	Average regeneration response of IK2, IK3 and IK4 on regeneration of wheat lines.....	100
Fig. 3.28	Cumulative regeneration pattern obtained at selected hormonal concentrations (IAA and Kinetin) against wheat cultivars. The vertical bars indicate the regeneration.....	100
Fig. 3.29	Ethidium bromide-stained agarose gel showing amplified coding region of <i>NDPK2</i> on 1% agarose gel. From right is 1 Kb DNA marker (M) and lane1 and 2 represent amplified coding region of <i>NDPK2</i> (544bp).....	103
Fig. 3.30	Colony PCR amplification of <i>NDPK2</i> gene found in p ^{CR8/GW/T-A} TOPO® cloning vector on 1% agarose gel. Lane M is 1 Kb DNA marker; remaining lanes represent colony PCR amplifications loaded on 1% agarose gel. Each lane is labeled with colony number.....	104
Fig. 3.31	Restriction digestion (BsrGI) of plasmid DNA, isolated from overnight cultures of <i>E. coli</i> carrying the pBRACT-214 destination vector, showed identical restriction patterns.....	104
Fig. 3.32	Schematic representation of pBRACT 214- <i>NDPK2</i> vector.....	105

Fig. 3.33	Response of different wheat cultivars on the first selection medium (a) Fielder showing a better and fast callus induction response during first selection followed by (b) Atta Habib (c) Marvi 2000 and (d) Siran.....	107
Fig. 3.34	Comparison of embryogenic callus formation at fourth week (second selection) (a) Fielder (b) Siran (c) Atta Habib and (d) Marvi 2000 (e) Iqbal 2000.....	108
Fig. 3.35	Response of wheat explants at fifth week on selection media shown under microscope (a) Fielder showing the growth of green shoots at the later stage of selection (b) Atta Habib and (c) Siran showing highly embryogenic calli with green spots (d) Marvi 2000 and (e) Iqbal 2000 with embryogenic callus.....	109
Fig. 3.36	Response of explants on Regeneration media (a) Fielder and (b) Siran with growth of green shoots after two week on regeneration medium.....	110
Fig. 3.37	Response of explants on regeneration medium (a) (a) Appearance of green shoots and strong roots developed in cv. Fielder (b) cv. Siran show an extensive root growth with the shoot formation.....	111
Fig. 3.38	Different stages of the wheat transformation: (a) sterilized wheat seeds before immature embryo isolation (b) an immature embryo after removal of embryonic axis (c) initiation of callus formation (d) embryogenic callus induction (e) initiation of green shoots on regeneration medium (f) plantlet rooting in glass culture before shifting to soil	112
Fig. 3.39	PCR amplified bands of <i>NDPK2</i> gene in barley plants regenerated following <i>Agrobacterium</i> inoculation. A negative (-) control of non-transgenic wild type plant DNA of cv Golden Promise (GP), molecular weight markers (M) (Promega Lambda DNA markers, 1 kb plus ladder), and the expected <i>NDPK2</i> PCR product (544 kb) are indicated. DNA samples used for the PCR reaction were taken from plants regenerated from immature embryos a. (1-13) and b. 14-22. Positive plants exhibiting bands of expected size.....	113
Fig. 3.40	PCR amplified bands of hygromycin gene in barley plants regenerated following <i>Agrobacterium</i> inoculation. A negative (-) control (non-transgenic plant DNA of cv Golden Promise), molecular weight markers (M) (Promega PCR markers 1-kb ladder), and the expected PCR NptII product (917bp) are indicated. DNA samples used for PCR reaction were taken from	

	plants regenerated from immature embryos a. (Lanes 1-13) and b. (Lanes 14-22). Positive plants exhibiting bands of expected size...	114
Fig. 3.41	Callus response during selection (a) highly embryogenic development during second selection. The black arrow heads are indicating the embryogenic nodes on the out surface of callus (b) Appearance of green shoots on its surface at the end of third selection.....	115
Fig. 3.42	Barley on regeneration mediumm (a) appearance of green shoots in the early phase of regeneration (b) emergence of strong roots later in regeneration.....	115
Fig. 3.43	Different stages during barley transformation (a) before inoculation arrangement of immature embryos (b) development of embryogenic callus from on callus induction medium (c) calli initiating shoot formation on transition stage (d) plantlets growth on regeneration medium (e) transformed barley showing rooting (f) Transgenic barley transferred to soil.....	116

LIST OF TABLES

Table 1.2	Top Ten Wheat Producer countries 2009 -2013 (million metric ton).....	8
Table 1.3	Top Ten Barley Producer countries 2011-2013 (million metric ton)	28
Table 2.1	Plant material used for callus induction.....	35
Table 2.2	Wheat Chromosomes substitution lines used for callus induction ...	40
Table 2.3	Composition and usage of culture media during wheat transformation.....	52
Table 2.4	Bacterial culture media.....	56
Table 2.5	Different media used during barley transformation.....	62
Table 3.1	Callus induction response of different wheat cultivars against each 2,4-D concentration	77
Table 3.2	Analysis of variance showing interaction between the cultivars and 2,4-D concentrations.....	77
Table 3.3	Callus induction response of different wheat lines against 2 and 3 mg/L 2,4-D concentrations.....	83
Table 3.4	Analysis of variance showing interaction between the wheat cultivars and varying concentrations of IAA and Kinetin.....	92
Table 3.5	Regeneration response of different wheat cultivars against each varying hormonal concentration.....	93
Table 3.6	Regeneration response of different wheat lines against varying concentrations of IAA and BAP.....	99
Table 3.7	Regeneration response of different wheat lines against varying concentrations of IAA and Kinetin.....	99
Table 3.8	Summary of transformation experiments conducted with varying wheat genotypes.....	106
Table 3.9	Summary of transformation experiments conducted with varying wheat genotypes.....	113

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
<i>NDPK2</i>	Nucleoside diphosphate kinase
MPK	Mitogen activated protein kinases
CAT	Catalase
Phy A	Phytochrome A
CDS	Coding DNA sequence
<i>E. coli</i>	<i>Escherichia coli</i>
BRAC	Biotechnology Resources for Arable Crop Transformation
JIC	John Innes Centre
Cv.	Cultivar
PCR	Polymerase Chain Reaction