

GENE MAPPING OF PHOSPHOGLYCEROL PHOSPHOLIPASE C HOMEOLOGS TO SINGLE CHROMOSOMES IN WHEAT (*Triticum aestivum* L.)

NESREEN SABRY¹, F. M. ABDEL-TAWAB² AND P. J. GULICK³

1. Benha University, Benha, Egypt
2. Ain-shams University, Cairo, Egypt
3. Concordia University, Montreal, Canada

Phospholipases are sets of enzymes which have the ability to hydrolyze phospholipids. They have different forms in plants; including phospholipases C, D and A. phospholipase C and D have important roles in signal transduction. The general structure of phospholipids consists of two fatty acyl chains esterified to a glycerol backbone at the sn-1 and sn-2 positions, and a phosphate at sn-3 position to which a variable head group (R) is attached. Phosphatidylglycerol plays a specific role in photosynthesis (Sakurai *et al.*, 2007) by helping in the organization and function of thylakoid membranes in plant cells.

Hagio *et al.* (2002) stated that the content of phosphatidylglycerol in both *Arabidopsis thaliana* mutants, that have an insertion in the PGP1 gene encoding a phosphatidylglycerol phosphate synthase, and wheat leaves of phosphate-deficient mutants were reduced than that of the wild type due to PGP1 gene disruption. Furthermore, the development of chloroplasts in the leaf cells was severely arrested in these mutants, evidence that PG is essential for the development of thylakoid membranes.

Most animals and many plant species are diploid, however many plant species are polyploid such as *T. aestivum*, bread wheat which is hexaploid. All members of the *Triticeae* tribe, including wheat, barley, and rye, have originated from a common ancestor.

Polyploid wheat species such as the tetraploid durum wheat *Triticum turgidum*, and the hexaploid bread wheat, *Triticum aestivum*, were formed by processes of interspecific hybridization events among different ancestral wheats followed by spontaneous chromosome doubling. The major genomes of wheat and its relatives, named A, B, D, G and S etc., have a basic chromosome number of seven ($X=7$) and within a polyploid species these genomes are referred to as being homeologous to one another. The polyploid wheat species constitute two evolutionary lineages. *T. turgidum* (AABB genome) and *T. aestivum* (AABBDD) provide one lineage, and *Triticum timopheevii* (AAGG) and *Triticum zhukovskiyi* (AAAAGG) constitute the other lineage. In the *T. aestivum* *T. turgidum* lineage, the A genome was contributed by *T. urartu* while the other lineage received the A genome from *Triticum*

monococcum. The B genome is thought to have been contributed by wild diploid wheat closely related to *Aegilops speltoides*. *Triticum aestivum* (AABBDD) arose from spontaneous hybridization between *T. turgidum* (AABB) and the diploid goat grass *Aegilops tauschii* (DD) (Faris *et al.*, 2001). Hexaploid bread wheat (*Triticum aestivum* L.) has the ability to tolerate the addition or loss of whole chromosomes or chromosome segments and this has contributed to the construction of several series of aneuploid lines. Nullisomic tetrasomic (NT) and ditelosomic lines have been used as tools for localization of molecular markers to chromosomes without the need of polymorphism at the gene level. In addition, 21 pairs of ditelosomic chromosome lines which have one pair of chromosomes being composed of either two identical short or long arms also were used to map genes to individual chromosome arms on the genetic maps. (Devos *et al.*, 1999).

Since bread wheat is a self-pollinated allohexaploid, most genes are expected to be present in three homeologous copies on three homeologous chromosomes derived from the three ancestral genomes; the alleles on homologous pairs of chromosomes are expected to be identical. Homeologous copies of genes from the A, B and D genome are expected to have very high nucleotide sequences similarity. For example, the three homeologous copies for each of the five paralogous gene copies of the α -tubulin gene family members in *T. aestivum* were found to be 97% identical

within the coding regions of the gene and to have somewhat greater divergence in the un-translated region of the gene transcript (Farajalla and Gulick, 2007).

The main objectives of this study were to determine the chromosomal location of the PLC genes in wheat and survey the prevalence of a frame shift mutation identified in one Ta-PG-PLC gene, while sequencing, among twenty-one wheat cultivars and in progenitor species.

MATERIALS AND METHODS

Triticum aestivum (L.) genetic stocks

Several accessions of diploid and tetraploid wheat species as well as several North American and Egyptian hexaploid *T. aestivum* cultivars were used in this study and are listed in Table (1). In addition, *T. aestivum* cytogenetic stocks were used for mapping PLC genes to individual chromosome arms. The nullisomic-tetrasomic (NT) lines have one chromosome pair substituted by a homeologous pair of chromosomes. The ditelosomic (DT) lines have single pairs of chromosomes which have two copies of one chromosome arm and are missing the other arm of the chromosome. Partial chromosome arm deletion lines are missing a part of one chromosome arm (Farajalla and Gulick, 2007); they were obtained from G. Scholes (University of Saskatchewan). The Egyptian cultivars which were used in this study were obtained from Plant Genetic Resources Dept., Desert Research Center, Egypt and the North American cultivars were ob-

tained from Agriculture and Agri-Food Canada (Table 1).

cDNA Clones

A cDNA clone for phosphatidylglycerol phospholipase Cs, Ta-PG-PLC2-1 was obtained from the Genome Canada Functional Genomics of Abiotic Stress (FGAS) program (Houde *et al.*, 2006) and Ta-PG-PLC2-2 was obtained from Arizona Genomics Institute (AGI). The clones were sequenced at the Genome Quebec Innovation Centre, McGill University. Additional partial cDNA clone of Ta-PG-PLC2-3 was assembled from EST sequences. Sequences were compared to the Rice gene sequences which obtained from GenBank using BLASTn and BLASTx. Homeologs genome assignment to ancestral genomes Gene specific primers were designed and used for PCR amplification with DNA from *Triticum urartu* (A genome progenitor), *Aegliops speltoides* (closely related to the B genome progenitor), *Triticum tauschii* (D genome progenitor) to determine the original genomes of the three PG-PLC genes. Specific primers are shown in (Table 2).

Nullisomic chromosomes mapping

The set of nullisomic-tetrasomic (NT) lines included 19 lines used for mapping the three ESTs homeologs to individual chromosomes (Farajalla and Gulick, 2007). Sequence differences between the homeologous genes were used to design gene specific primers. PCR reactions were conducted using gene

specific left and right primers. The primer sequences are shown in (Table 2).

The seven A, B and D genome nullisomic-tetrasomic (NT) lines were used to determine the chromosomal number of Ta-PG-PLC2-1, Ta-PG-PLC2-2 and Ta-PG-PLC2-3 homeologs, respectively. Amplification was carried out in a Applied Bio-systems GeneAmp® PCR system 9700 and a touchdown PCR program was used and programmed as follows: 95°C/30 Sec (1 cycle), 95°C/30 Sec, 67°C/30 Sec, 72°C/1 min (3 cycle), 95°C/30 Sec, 65°C/30 Sec, 72°C/1 min, (3 cycle), 95°C/30 Sec, 62°C/30 Sec, 72°C/1 min, (3 cycle), 95°C/30 Sec, 59°C/30 Sec, 72°C/1 min (25 cycles); 72°C/4 min (1 cycle) and 4°C (hold).

Screening for the prevalence of a mutation in Ta-PG-PLC2

One pair of primers was designed to detect the two base pair deletion mutation detected in Ta-PG-PLC2-1. Another left primer was designed to detect a putative wild type version of Ta-PG-PLC2-1 (reverted) without the two bp deletion, and additional pair of primers was designed to detect the homeologous gene Ta-PG-PLC2-2. The primers sequences are shown in (Table 3). For Ta-PG-PLC2-1 primers were designed to amplify the PLC gene from twenty one wheat cultivars from different origins (Table 1) to determine the prevalence of the frame shift mutation.

Amplification was carried out in a Biometra T- Gradient thermocycler programmed as follows: 94°C/3 min (1 cycle), 94°C/30 Sec, 65°C/35 Sec, 72°C/2 min (40 cycles); 72°C/10 min (1 cycle) and 4°C (hold).

RESULTS AND DISCUSSION

Wheat PLC gene sequence analysis

Two Ta-PG-PLC clones (Ta-PG-PLC2-1 and Ta-PG-PLC2-2) were sequenced.

Ta-PG-PLC2-1 Sequence

Ta-PG-PLC2-1 clone is 1734 bp in length (Fig. 1) with a 1568 bp open reading frame extending from position 61 to position 1629. The wheat sequences have 87% nucleic acid and 83% amino acid sequence similarity with the rice phosphatidylglycerol specific phospholipase C, Os03g0852800. The cDNA has a full length sequence similarity to its rice ortholog, beginning with the first ATG in the cDNA sequence. However, Ta-PG-PLC2-1 has a frame shift mutation, a deletion of two bases following the G at position +30 relative to the first ATG. This deletion is seen by sequence comparison to the rice phosphatidylglycerol specific phospholipase C gene and by comparison to an apparent homolog from wheat, as described below (Figs 2, 3 and 4). The apparent mutation was confirmed by the fact that the sequence quality is excellent in this region, a FRED score of 20 or more indicates higher than 99% sequence accuracy, and two independent sequences con-

firmed the DNA sequence in this region. The apparent frame shift is followed by second ATG 28 nucleotides downstream of the frame shift that is in-frame with the major ORF that may serve as the start codon (Fig. 1).

Gene mapping to single chromosomes

Nullisomic-tetrasomic (NT) analysis

The three cDNA clones for Ta-PG-PLC had 93% nucleotide sequence identity within their coding regions and were hypothesized to be homologous copies of the gene, since similar degrees of identity were seen among the homologs of alpha-tubulins in wheat (Farajalla and Gulick, 2007). Cytogenetic stocks of hexaploid wheat cultivars Chinese Spring (*T. aestivum* L.) were used for chromosomal mapping of gene family members. Cytogenetic DNA stocks were screened by PCR with gene specific primers for each homolog. A primer set that was specific to Ta-PG-PLC 2-1 that could detect the 2 nt deletion amplified fragments in *T. urartu* (the A genome progenitor), *T. trugidum* and *T. aestivum* but not in *Aegilops speltoides* or in *Aegilops tauschii*, the B and D genome relatives. This indicated that Ta-PG-PLC2-1 is likely in the A genome (Fig. 5). Further PCR screening of the A genome nullisomic-tetrasomic (NT) chromosome lines 1-7 A, ditelosomic DT6AS and DT6aL, and selected chromosome deletion lines indicated that Ta-PG-PLC2-1 is on the short arm of chromosome number 6A. Ta-PG-PLC2-2 and Ta-PG-PLC2-3 gene specific primers amplified products from *A. speltoides*, the B genome progeni-

tor and *A. tauschii* (D genome progenitor), respectively (Fig. 5). Further analysis with NT, DT and chromosome deletion lines indicated that Ta-PG-PLC2-2 homeolog is on the long arm of chromosome 6B and Ta-PG-PLC2-3 homeolog is on the short arm of chromosome number 6D (Fig. 6).

The presence of Ta-PG-PLC2-1 and Ta-PG-PLC2-3 was present on the short arms of A and D chromosome, respectively, whereas Ta-PG-PLC2-2 was present on the long arm of B chromosome may be due to duplication and then elimination or silence of the gene hence in the wheat evolution early stages genes may be duplicated and then they may be eliminated or silenced during domestication. Qi *et al.* (2004) found that from 5762 ESTs, 1086 hybridized to restriction fragments on chromosomes in different homologous groups. Within this group (75%) detected loci were in two homologous groups, 17% detected loci in three groups, and 10% were detected loci in more than three groups. Like BE443755 which hybridized to 17 fragments that could be located at 1A, 5A, all B-genome chromosomes, and 7D. Nine loci of those were mapped on opposite arms within homologous groups on chromosomes 1AL 1BS and 5AS- 5BL and on both arms of chromosomes 4B and 7B. In several cases of intrachromosomal duplications, the duplicated fragments were detected in both arms. These events may represent peculiarities of chromosome behavior during which these duplications occurred. For example, opposite arms may be aligned together during prophase of meiosis, giving opportunities for

gene-conversion to happen. When these duplicated genes may be eliminated, and/or silenced, fragment would be mutated to give new functions, or epigenetically regulated for tissue-specific expression (Adams *et al.*, 2003). This is a reasonable explanation for the presence of two homeologs on short arm chromosomes and the third one on the long arm.

Mutation detection in different wheat cultivars

Twenty one wheat cultivars were tested to detect the 2 nt deletion found in Ta-PG-PLC2-1 (Table 1). The specific primers aforementioned were designed to characterize the region of this mutation in the three wheat ancestral genomes of hexaploid wheat, *T. urartu* (A genome), *A. speltoides* (B genome), *A. tauschii* (D genome), *T. turgidum* (AB genome) and *T. aestivum L* (ABD genomes) (Fig. 7). PCR primers were designed to amplify the Ta-PG-PLC2-1 sequence containing the deletion mutation amplified products from *T. urartu*, *T. turgidum* and *T. aestivum L*. The Ta-GP-PLC2-1 primer pair amplified PCR products in all *T. aestivum* cultivars that were screened. A modified Ta-GP-PLC2-1 primer pair in which the two nucleotide sequences were reinserted failed to amplify any PCR products in *T. aestivum* cultivars or in related wheat species. From these results it was apparent that the mutation is widespread in wheat plant and it is quite clear that it is not unique to polyploid wheat but it is also present in its ancestral species (Fig. 8). This also suggests that the gene is func-

tional and that the 2 nt deletion may impart a novel function to the gene since its persistence would appear to be under positive selection.

In Summation phospholipase C gene family plays a crucial role in signal transduction as well as in changes in membrane lipid composition. The two full length wheat PLCs cDNA clones were sequenced and related wheat PLC sequences identified in the GenBank data base and characterized. Homeologs to one of the wheat full length clones, Ta-PG-PLC2, with 93% nucleotide sequence identity were identified. The origins of the three members of the PG-PLC gene family in the ancestral genomes of *T. aestivum* were suggested by cytogenetic analysis, i.e. by both nullisomic- tetrasomic and ditelosomic analysis coupled with the amplification with gene-specific primers for each homolog of each bread wheat ancestors (*T. urartu*, *A. speltoides* and *A. tauschii*). In addition, a frame shift mutation was found in genome A progenitor and in all twenty one bread wheat cultivars. This mutation likely provided some positive selective advantage, its identification in the diploid progenitor suggests that it is an old mutation. If the gene were not functional, we might expect the gene to accumulate other mutations, but the cDNA suggests an intact and functional gene. In relation to the diploid wheat, other members of the gene family may compensate for the change of function of this gene family member.

SUMMARY

Phospholipase Cs (PLCs) has been recognized as important enzymes for their roles in regulation, signal transduction and membrane composition. Two full length wheat PLCs cDNAs, Ta-PG-PLC2-1 and Ta-PG-PLC2-2 from wheat, *Triticum aestivum* L., were sequenced and mapped to wheat chromosome arms using PCR amplification in diploid wheat ancestors, *Triticum urartu*, *Aegilops speltoides*, *Aegilops tauschii* and cytogenetic stocks of *Triticum aestivum* and found to have very high sequence similarity and one, PG-PLC2-1, was found to contain a frame shift mutation 29 nt downstream of the first ATG start codon. This sequence was used to survey the prevalence of the mutation in twenty one wheat cultivars and was found to be present in all wheat cultivars tested and in the ancestral diploid species, *Triticum urartu*.

REFERENCES

- Adams, K. L., R. Cronn, R. Percifield and J. F. Wendel (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proc. Natl. Acad. Sci. USA*, 100: 4649-4654.
- Devos, K. M., M. E. Sorrells, J. A. Anderson, T. E. Miller, S. M. Reader, A. J. Luckaszewski, J. Dubcovsky, P. J. Sharp, J. Faris and M. D. Gale (1999). Chromosome aberration in wheat nullisomic-tetrasomic and

- ditelosomic lines. Cereal research communications, 273: 231-239.
- Farajalla, M. R. and P. Gulick (2007). The α -tubulin gene family in wheat (*Triticum aestivum* L.) and differential gene expression during cold acclimation. Genome; 50: 502-510.
- Faris, J., A. Sirikhachornkit, R. Haselkorn, B. Gill and P. Gornic (2001). Chromosome mapping and phylogenetic analysis of the cytosolic acetyl-coa carboxylase loci in wheat. Mol. Biol. Evol., 18: 1720-1733.
- Hagio, M., I. Sakurai, S. Sato, T. Kato, S. Tabata and H. Wada (2002). Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. Plant Cell Physiol., 43: 1456-1464.
- Houde, M., M. Belcaid, F. Ouellet, J. Danyluk, A. Monroy, Ani Dryanova, P. Gulick, Anne Bergeron, A. Laroche, M. Links, L. Macarthy, W. Crosby and F. Sarhan (2006). Wheat EST re-sources for functional genomics of abiotic stress. BMC Genomics, 7: 149-170.
- Qi, L. L., B. Echaliier, S. Chao, G. R. Lazo, G. E. Butler, O. D. Anderson, E. D. Akhunov, J. Dvor[□]a[□]k, A. M. Linkiewicz, A. Ratnasiri, J. Dubcovsky, C. E. Bermudez-Kandianis, R. A. Greene, R. Kantety, C. M. La Rota, J. D. Munkvold, S. F. Sorrells, M. E. Sorrells, M. Dilbirligi, D. Sidhu, M. Erayman, H. S. Randhawa, D. Sandhu, S. N. Bondareva, K. S. Gill, A. A. Mahmoud, X.F. Ma, Miftahudin, J. P. Gustafson, E. J. Conley, V. Nduati, J. L. Gonzalez-Hernandez, J. A. Anderson, J. H. Peng, N. L. V. Lapitan, K. G. Hos-sain, V. Kalavacharla, S. F. Kianian, M. S. Pathan, D. S. Zhang, H. T. Nguyen, D. W. Choi, R. D. Fenton, T. J. Close, P. E. McGuire, C. O. Qualset and B. S. Gill (2004). A chromosome bin map of 16,000 expressed sequence Tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics, 168: 701-712.
- Sakurai, I., N. Mizusawa, S. Ohashi, M. Kobayashi and H. Wada (2007). Effects of the lack of phosphatidylglycerol on the donor side of photosystem II. Plant Physiol., 144: 1336-1346.

Table (1): Wheat species and cultivar's used to survey mutation in PG-PLC2.

Wheat cultivar's Name	Genome type	Wheat's accessions numbers or commercial name
1- <i>T. urartu</i>	A	CN 34145
2- <i>T. urartu</i>	A	PGR 11079
3- <i>A. speltoides</i>	B	CN 30945
4- <i>A. speltoides</i>	B	CN 108019
5- <i>T. turgidum</i>	AB	CN 32492
6- <i>T. turgidum</i>	AB	CN 1841
7- <i>T. turgidum</i>	AB	CN 33585
8- <i>A. tauschii</i>	D	CN 30803
9- <i>A. tauschii</i>	D	CN 30941
10- <i>T. aestivum</i>	ABD	CN 11995
11- <i>T. aestivum</i>	ABD	CN 2767
12- <i>T. aestivum</i>	ABD	CN 11057
13- <i>T. aestivum</i>	ABD	AMZA 0115611
14- <i>T. aestivum</i>	ABD	Norstar
15- <i>T. aestivum</i>	ABD	Bounty
16- <i>T. aestivum</i>	ABD	Sahel1
17- <i>T. aestivum</i>	ABD	Sohag3
18- <i>T. aestivum</i>	ABD	Giza 157
19- <i>T. aestivum</i>	ABD	Giza 160
20- <i>T. aestivum</i>	ABD	Sakha 69
21- <i>T. aestivum</i>	ABD	Sakha 61

Table (2): Specific primers used for Mapping Ta-PG-PLC2 homeologs in wheat.

Primer	Sequence orientation	Primer's Sequence	Melting Temperature	G+C % ratio
Ta-PG-PLC2-1	Right	5`-CCATGTACGTTTTGGGACA-3`	57.79	47.37
Ta-PG-PLC2-1	Left	5`-GGAGCACACGACGATCTC-3`	57.72	61.11
Ta-PG-PLC2-2	Right	5`-CCAGTTTATCTATCTTTTTGGGACA-3`	59.80	36.00
Ta-PG-PLC2-2	Left	5`-GCGTCCATCTCGGACTTC-3`	59.30	61.11
Ta-PG-PLC2-3	Right	5`-CCCCAACGCATGTATCCT-3`	59.32	55.56
Ta-PG-PLC2-3	Left	5`-GCGACGATCTCGGACTTC-3`	59.46	61.11

Table (3): Primers used for mutation survey in wheat.

Primer	Sequence orientation	Primer's Sequence	Melting Temperature	G+C % ratio
Ta-PG-PLC2-2	Left	5'-AGTGGTGGTGGTGGTGCAG-3'	63.18	63.16
Ta-PG-PLC2-2	Right	5'-TGCGTGTGTTGTTGCTCACC-3'	60.47	55.56
Ta-PG-PLC2-2	Left	5'-GGTGGTGGTGGTTGCAAG-3'	61.02	61.11
Ta-PG-PLC2-1	Right	5'-CCTTGGTGTGTTGCTGACG-3'	62.69	55.00
Ta-PG-PLC2-1 reverted	Left	5'-GGTGGTGGTGGTGGTGGTCAA-3'	66.42	63.16

AAGACGCGGGCCGAGCTATCGATCGGGAGATCAAGAGCAAGCAACTAGCGATCGAAA**ATG**
GCGGCCAAGATCAAGACGGTGGTGGTGG**TT**GCAAGAGAACCGCTCCTTCGACCAC**ATG**CT
GGGCTGGATGAAATCCCTCAACCCGGACATCGACGGCGTCACCGGCGCCGAGACTAACCG
CGTCGTCGTTGCCGACCCCCGCTCCAAAGCCATCCCCTTCGGCGACGGCTCCCAGTACGTG
GACCCGGACCCCGGCCACTCCATCCAGGCCATCTACGAGCAGGTCTATGGCACGCCCTCG
TCGACGCCAGGCCACGCCATCACCCCCGCCAGCGTCCCCACGCCCCATGAGCGGTTT
CGCGCAGCAGGCCGAGCAGGAGAAGCCGGGCATGGCGGAGACCGTCATGAGCGGGTTTCAG
GCCGGACGCCGTGCCGGTGTACCGGAGCTGGCGGGAGTTCGCCGTGTGCGACCGCTGG
TTCGCGTCAACCCGGCGTCCACCCAGCCCAACCGCCTTTCGTGCACTCGGCCACCTCGCA
CGGCTCTGTCAGCAACAACACCAAGGCGCTCGTCGCCGGCCTGCCGCAGACCACCATCTTC
GACTCCCTCCACGACGCCGGCTACTCTTCGGGATCTACTACCAGTACCCGCCATCCGTGCT
CTTCTACCGCAACCTCCGGCAGCTCAAGTACATCGGCAACTTTCACCCGTTTCGACTCGAGT
TCCGGCGCCACTGCCGGGAGGGGAAGCTGCCAAACTATGTCGTCGTTGGAGCAGCGCTACTT
TGACCTAAGATCCTCCCCGCAACGACGACACCCCGTGCATGACGTTTCCGAGGGCCAG
AGGTTTCGTAAGGAGGTGTACGAGGCGCTGCGCTCGGGCCCCGAGTGGGAAGAGACGCTC
CTTGTGCTCACCTACGACGAGCAGCGCGGGTTCATGACCACGTGCCACCCCGTGGGCG
TCCCCAGCCCCGACGACATCGTCAGCGACGCACCCCTTCTTCTCAACTTCGACCCCTCGGC
GTCCGCGTCCCGGCTTCTTCATCTCCCCCTGGATCGAGCCTGGGACGGTGGTGCACAGGC
CGTCAGGGCCGCACCCGACATCGGAGTTCGAGCACTCGTCCATCCCGGCCACCGTGAAAAA
GATCTTCAACTCGAGAGCTTCTTGACCAAGCGCGACGCATGGGCCGGCACGTTTCGACGTC
GTCCTTACGCGTGACACCCCGCGCACCGATTGCCCTGCCACGCTGCCCTGAGCCGGTGAAGA
TGCGGCCAACAGAGGCAGCGGAGCACACGACGATCTCGGACTTCCAGGCGGAGCTGGTGC
AGCTGGGCGCGGCTCAACGGCGACCACGCCAAGGACACATACCCACACAAGCTGGTGG
AGGGCATGACGGTGGCGGACGCCGCAAGGTAAGTACTGCAACGGCGCCTTCAAGGCCTTCCAGG
ACGAGTGCAGCGCTGCAAGAAGTGGCGCAAGGATGGGTCCCACATCCCCACCATGCCGC
CGTCGCCGTTGCCCGCTCCAAGAAGAGGGGTGGCTTCGCTTCCAAGATGCTGGCTGCTT
TGCGTGTGGCCATTCC**TAG**GATTGCTGGTTGGATTGTTTGGTGGGAATAGTTGGACTGGTT
GGTTGCTTACGATAGCTAAGATTTGGTATGCTCATGTCCCAAAACGTACATGGGGTGT
GGTAATGATTGAACGTGTGAATTTCTACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. (1): Ta-PG-PLC2-1, potential start and stop codons are underlined and in bold. There is a two nt deletion relative to wheat gene Ta-PG-PLC2-1 and rice gene Os03g0852800 between the two bolded T's.


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CLUSTAL 2.0.11 multiple sequence alignment

Ta-PG-PLC2-1
AAGACGCGGGCCGAGCTATCGATCGGGAGATCAAGAGCAAGCAACTAGCGATCGAAAA-- 58
oryza      -----ATGGC-
GGCGGCGGGCCGC-- 17
Ta-PG-PLC2-2      -----
GCACGAGGCTAAAGATCAGTAGCAAGCAGCTAGCAGCTGGCTATAGAAATAA 52
Ta-PG-PLC2-3      -----
-----

Ta-PG-PLC2-1      TGGCGGCCAAGATCAA-GACGGTGGTGGTGGTTGCAA--
GAGAACCGCTCCTTCGACCAC 115
oryza      CGGCGGC-AAGATCAA-
GACGGTGGTGGTGGTGGTGATGGAGAACCGCTCCTTCGACCAC 75
Ta-PG-PLC2-2      TGGCGGCCAAGATCAA-
GACAGTGGTGGTGGTGGTGCAGGAGAACCGCTCGTTTCGACCAC 111
Ta-PG-PLC2-3      -----AGGGGCCAACGGAGGCGGCGGAGCACGCGA-----
CGATCTCGGACTTCC 45

                                *  * * * *  *  * * * *  *  * * * *
*
Ta-PG-PLC2-1
ATGCTGGGCTGGATGAAATCCCTCAACCCGGACATCGACGGCGTCACCGGCGCCGAGACT 175
oryza
ATGCTGGGCTGGATGAAGTCTCTCAACCCGGAGATCGACGGCGTCACCGGCGACGAGATC 135
Ta-PG-PLC2-2
ATGCTGGGCTGGATGAAATCCCTCAACCCGGACATCGACGGCGTCACCGGTGCCGAGACC 171
Ta-PG-PLC2-3      AGGCGGAGCTGG-TGCAG---
CTAGGCGGGCGCTCAACGGCGACCACGCCAAGGACACG 101
                                *  * * * * * * *  *  * * * *  *  * * * *  *  *
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Fig. (4): Part of ClustalW alignment between the three Ta-PG-PLC2 homeologs and the rice gene Os03g0852800. Ta-PG-PLC2-1, 2-2, 2-3 and the *Oryza sativa*. The start codons are highlighted and underlined.

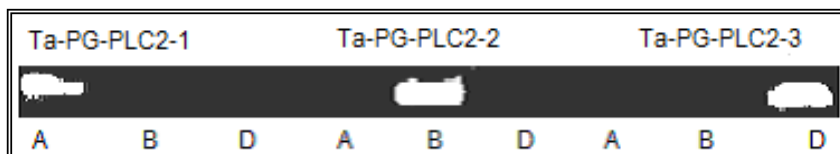


Fig. (5): Detection of the three PG-PLC2 homeologs in different wheat species, A is *T. urartu*, B is *A. speltoides* and D is *A. tauschii*.

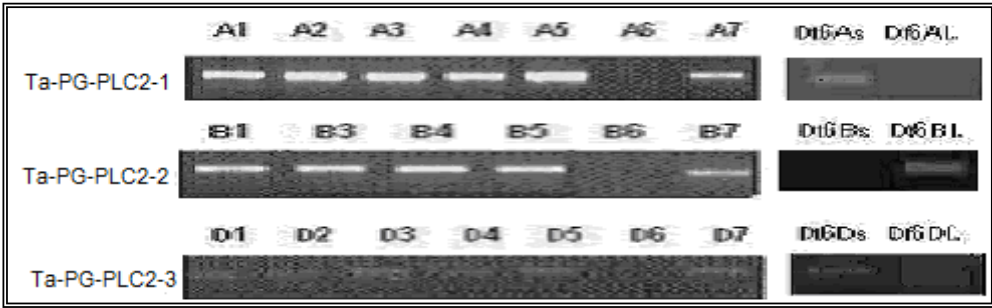


Fig. (6): Nulli-tetra lines on the left, the ditelo figures on the right 6D on Ta-PG-PLC2-3 Dt6Ds and Dt6Ds, Ta-PG-PLC2-2 DT6BL and Ta-PG-PLC2-1 Dt6As.

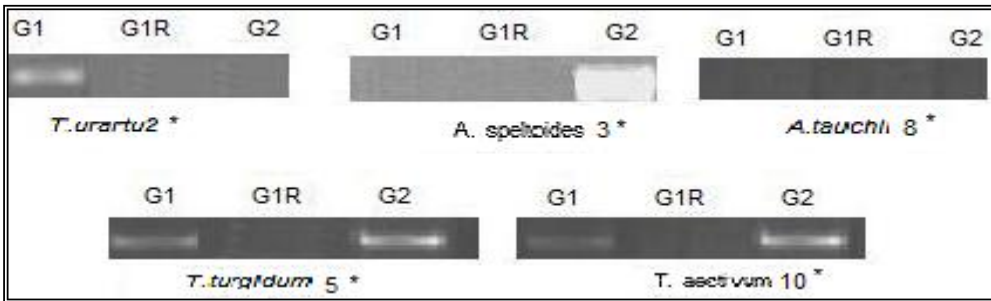


Fig. (7): Screening of the Ta-PG-PLC2-1 mutation in the three genomes.
* Check Table (3), G1 primer pair corresponds to Ta-PG-PLC2-1, G1R is Ta-PG-PLC2-1 reverted by the addition of 2 nt and G2 is Ta-PG-PLC2-2.

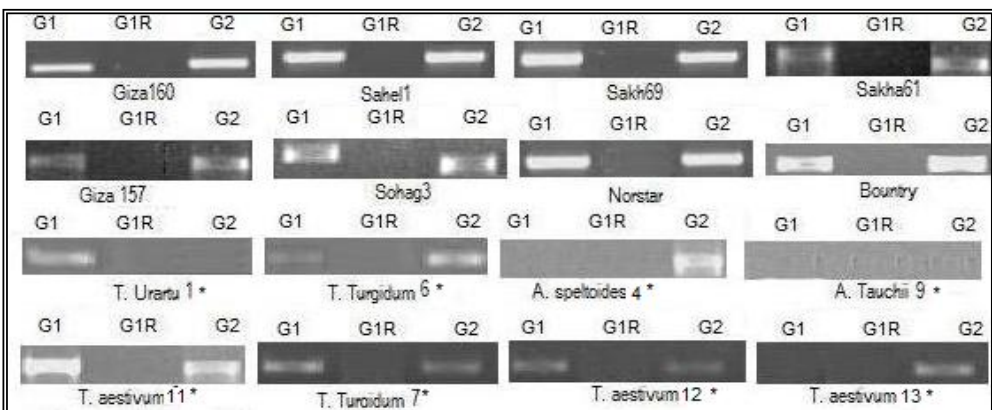


Fig. (8): Screening of the Ta-PG-PLC2-1 mutation in different wheat cultivars.
* G1 is Ta-PG-PLC2-1, G1R is Ta-PG-PLC2-1 reverted and G2 is Ta-PG-PLC2-2. The numbers referred to the accessions numbers in table (1). Samples listed by cultivar's name are *T. aestivum*.