Agri Gene 7 (2018) 43–51

Contents lists available at ScienceDirect

Agri Gene

journal homepage: www.elsevier.com/locate/aggene

Identification of *BABY BOOM* homolog in bread wheat

Andriy Bilichak*, Justin Luu, Fengying Jiang, François Eudes

Lethbridge Research and Development Center, Agriculture and Agri-Food Canada, Canada

ARTICLE INFO

Keywords:
BABY BOOM
Wheat
Embryogenesis
Microspores
Double haploids

ABSTRACT

Modern breeding practice of small grain cereals necessitates the development of an efficient system for the large scale and reproducible production of the doubled haploid (DH) lines. It is believed that among the available DH generation techniques, only isolated microspore culture (IMC) can satisfy the demand of public and private breeding programs. Unfortunately, the IMC method is prone to several challenges that jeopardizes its large scale adoption. One of the approaches to limit the variation in DH plant production and increase the efficiency of the method is manipulation of embryogenesis-related genes. Here we set up a study to map *BABY BOOM* in a bread wheat genome. The gene is one of the morphogenic regulators of somatic embryogenesis in plants. To achieve this task, we used amino acid sequences of *Zea mays* BBM-like proteins. *TaBBM* homoeologs were mapped to chromosomes 6AL, 6BL and 6DL. Amino acid sequence analysis revealed the presence of two AP2 domains and bbm-1 motif in the A and D copies and only one AP2 domain and bbm-1 motif in the B copy. This, along with the absence of both gene expression and predictable TATA-box, suggests that *TaBBM-gB* is a pseudogene. The expression pattern of the identified A and D homoeologs was similar to that for the *BBM*-like genes in other species and presence of the transcript was detected in an embryogenic microspore population. Identification of the *TaBBM* homolog can have application in elevating the efficiency of DH production, tissue culture, plant transformation and genome editing for wheat improvement.

1. Introduction

Androgenesis through isolated microspore culture (IMC) is an example of an unorthodox embryogenesis pathway in both monocots and dicots (Zheng, 2003; Ferrie and Caswell, 2011). The microspores are haploid predecessors of male gametes that, under certain conditions, have a unique ability to undergo embryogenesis with subsequent production of fully developed green haploid or doubled haploid (DH) plants. The IMC has an important application in the modern breeding practice, since it allows generation of recombinant but fully homozygous lines in a shorter period of time as compared to more conventional breeding methods (Germanà, 2011). In most of the plant species where the IMC was established, the microspores become competent to form embryos around the first mitotic division (pollen mitosis I). The cells at this stage are characterized by having a large vacuole and peripherally located nucleus (Soriano et al., 2013). One of the first changes of the microspore architecture that has been observed to occur following stress treatment is the reorganization of the cytoskeleton, movement of the nucleus to the center of the cell and formation of a plane of division that is usually absent during normal pollen development (Telmer et al., 1993; Simmonds and Keller, 1999). The rearrangement of the cytoskeleton results in a star-like morphology with the central nucleus and cytoplasmic strands protruding away to the periphery (Gervais et al., 2000). Although, the star-like appearance of the cytoplasm has been described as the first sign of embryogenic induction (Maraschin et al., 2005) there exists conflicting evidence as to

Abbreviations: TaBBM, *Triticum aestivum BABY BOOM*; AP2, APETALA 2 domain; IMC, isolated microspore culture; DH, double haploid; CPP, cell penetrating peptide; *LEC1*, *LEAFY COTYLEDON1*; *LEC2*, *LEAFY COTYLEDON2*; *PKL*, *PICKLE*; *SERK*, *SOMATIC EMBRYOGENESIS RECEPTOR KINASE*; *AGL15*, *AGAMOUS-like 15*; *FUS3*, *FUSCA3*; *STM*, *SHOOT MERISTEMLESS*; *WUS*, *WUSCHEL*; AP2/ERF, APETALA 2/ETHYLENE RESPONSE FACTOR; ANT, AINTEGUMENTA; euANT, eudicotANT; PLT, PLETHORA-like; CDS, coding sequence; ASGR, apospory-specific genomic region; DOC, days of culture; A.tau, *Aegilops tauschii*; A.com, *Ananas comosus*; A.off, *Asparagus officinalis*; B.vul, *Beta vulgaris*; B.dis, *Brachypodium distachyon*; C.caj, *Cajanus cajan*; C.ann, *Capsicum annuum*; C.can, *Coffea canephora*; C.mel, *Cucumis melo*; C.sat, *Cucumis sativus*; D.car, *Daucus carota*; D.ol, *Dichanthelium oligosanthe*; E.guin, *Elaeis guineensis*; E.pus, *Erycina pusilla*; G.max, *Glycine max*; G.soja, *Glycine soja*; G.arb, *Gossypium arboretum*; G.raim, *Gossypium raimondii*; G.hir, *Gossypium hirsutum*; H.vul, *Hordeum vulgare*; J.cur, *Jatropha curcas*; J. reg, *Juglans regia*; L.ang, *Lupinus angustifolius*; M.esc, *Manihot esculenta*; M.not, *Morus notabilis*; M.ac, *Musa acuminata*; N.nuc, *Nelumbo nucifera*; N.att, *Nicotiana attenuate*; N.tab, *Nicotiana tabacum*; N.tom, *Nicotiana tomentosiformis*; N.syl, *Nicotiana sylvestris*; O.br, *Oryza brachyantha*; O.sat, *Oryza sativa*; P.vul, *Phaseolus vulgaris*; P.dac, *Phoenix dactylifera*; P.eup, *Populus euphratica*; P.trich, *Populus trichocarpa*; R.com, *Ricinus communis*; S.ind, *Sesamum indicum*; S.it, *Setaria italic*; S.lyc, *Solanum lycopersicum*; S.tub, *Solanum tuberosum*; S.bic, *Sorghum bicolor*; T.cac, *Theobroma cacao*; T.ur, *Triticum urartu*; V.mon, *Vernicia montana*; V.ang, *Vigna angularis*; V.rad, *Vigna radiata*; V.vin, *Vitis vinifera*; Z.mays, *Zea mays*; Z.mar, *Zostera marina*; Z.juj, *Ziziphus jujuba*; DPA, days post anthesis; QTL, quantitative trait loci

* Corresponding author at: Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4P4, Canada.
E-mail address: andrii.bilichak@agr.gc.ca (A. Bilichak).

https://doi.org/10.1016/j.aggene.2017.11.002
Received 12 May 2017; Received in revised form 14 September 2017; Accepted 17 November 2017
Available online 21 November 2017

whether it can be considered a reliable marker of embryogenesis (Daghma et al., 2012; Zur et al., 2013). Similarly, it has been suggested previously that an initial symmetric division of the microspore cell is an indicator of the cell entering embryogenesis (Indrianto et al., 2001; Pulido et al., 2005). Nonetheless, this notion did not find strong support in studies conducted in monocots and dicots (Barnabás et al., 1999; Tang et al., 2013).

Lack of definitive morphological markers for embryogenesis induction necessitates the identification of the gene markers for the early switch in the microspore population development from gametophyte to sporophyte. The molecular markers being universal in their nature can be adapted to the number of new species and genotypes where the IMC is just being established. This is especially important since the IMC has a negative characteristic of being highly genotype dependent. Furthermore, manipulation of the embryogenesis-related genes by either downregulation through RNAi or upregulation via supplementing of the proteins using cell penetrating peptide (CPP) technology (Bilichak, Luu et al. 2015) can aid in improvement of the IMC for the DH production. Some of the genes that have been identified to play a key role in the establishment and maintenance of the embryogenesis-related state include *LEAFY COTYLEDON1* (*LEC1*), *LEAFY COTYLEDON2* (*LEC2*) (Meinke et al., 1994; West et al., 1994), *PICKLE* (*PKL*) (Ogas et al., 1999), *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*SERK*) (Hecht et al., 2001), *AGAMOUS-like 15* (*AGL15*) (Heck et al., 1995), *FUSCA3* (*FUS3*) (Gazzarrini et al., 2004), *SHOOT MERISTEMLESS* (*STM*) (Elhiti et al., 2010), *WUSCHEL* (*WUS*) (Zuo et al., 2002) and *BABY BOOM* (*BBM*) (Boutilier et al., 2002). Among the aforementioned genes only three – *WUS*, *BBM* and *LEC* do not require exogenous application of plant hormones for somatic embryogenesis when overexpressed in transgenic plants.

A *BBM* transcript was originally isolated as a cDNA clone from the microspore-derived embryos of *Brassica napus* at the 8 to 32 cell stage of development (Boutilier et al., 2002). The gene encodes a transcription factor and is a member of the APETALA 2/ETHYLENE RESPONSE FACTOR (AP2/ERF) DNA-binding domain superfamily that has been identified in the number of plants including gymnosperms, angiosperms as well as algae and mosses (Kim et al., 2006). The family contains the AP2-like genes with either single or tandem AP2 domains comprised of 60–70 amino acid residues separated by a linker region, respectively (Sakuma et al., 2002). The AP2 domain is also referred to as the ethylene-responsive element-binding factor domain (ERF domain), since it can bind to the GCC box, a DNA sequence involved in ethylene response (Ohme-Takagi and Shinshi, 1995). In relation to the number of the AP2 domains and presence of additional motifs in the protein sequence, the AP2/ERF superfamily is divided into 5 classes: AP2 subfamily, RAV subfamily, DREB subfamily, ERF subfamily, and others (Sakuma et al., 2002). The AP2-like clade contains two lineages – eudicotAP2 and AINTEGUMENTA (ANT). The latter is further divided into basalANT and eudicotANT (euANT) lineages (Kim et al., 2006). Apart from the BBM-like subgroup, the euANT lineage includes the PLETHORA-like (PLT), AINTEGUMENTA-like, AINTEGUMENTA-like1 and AINTEGUMENTA-like5. The distinct feature of the BBM and BBM-like proteins is the presence of a conserved bbm-1 motif (GLSMIKTW) that is absent in the other proteins of the euANT lineage. An importance of the bbm-1 domain was demonstrated in deletion experiments where Arabidopsis plants overexpressing *BBM* gene with a mutated bbm-1 domain failed to produce somatic embryos on cotyledons; a characteristic feature of transgenic plants bearing the complete CDS of the *BBM* transgene (El Ouakfaoui et al., 2010). Overall, members of the AP2 superfamily play a vital role in the range of processes related to plant growth, reproduction and interaction with the environment (Riechmann and Meyerowitz, 1998; Feng et al., 2005; Nole-Wilson et al., 2005). Furthermore, in a recent study, members of the BBM-like lineage have been linked to propagation in *Pennisetum/Cenchrus* species (grass family) through apomixis – asexual reproduction in flowering plants that does not require meiosis or egg fertilization for seed production (Conner et al., 2015). The apomixis trait in *Pennisetum squamulatum* is encoded by the apospory-specific genomic region (ASGR) that contains multiple copies of the *PsASGR-BABY BOOM-like (PsASGR-BBML)* gene. Expression of the *PsASGR-BBML* transgene in pearl millet that normally demonstrates sexual reproduction resulted in both development of haploid offspring and parthenogenesis (embryo formation without fertilization). Therefore, it has been suggested that utilization of the *PsASGR-BBML* locus can find its application in the other cereal crops both for induction of apomixis and production of haploids to rapidly advance breeding through fixing a trait in the DH plants (Conner et al., 2015).

An important feature of the *BBM* gene in embryogenesis was also shown in *Arabidopsis* and *B. napus*, where its ectopic overexpression induces formation of somatic embryos (Boutilier et al., 2002). At the same time, constitutive overexpression of the gene causes many pleiotropic effects and altered plant morphology during plant development. Curiously, overexpression of a *BnBBM* gene in transgenic tobacco resulted in altered plant phenotype without prominent somatic embryogenesis (Srinivasan et al., 2007). Similarly, the overexpression of two *BBM* genes from *Rosa canina* in *Arabidopsis* increased the shoot regeneration in tissue culture, but did not enhance spontaneous somatic embryogenesis (Yang et al., 2014). Therefore, this suggested that the *BBM* proteins from different species are not always able to integrate into the embryogenesis pathway of a distinct dicot. Overexpression of the *BBM* gene to enhance somatic embryogenesis was adopted in a number of species including cacao *Theobroma cacao* (Florez et al., 2015), sweet pepper *Capsicum annuum* (Heidmann et al., 2011), maize, rice, sorghum, and sugarcane varieties (Lowe et al., 2016). More importantly, in addition to an increase in somatic embryogenesis, tissues with the overexpressed *BBM* gene become more competent to stable *Agrobacterium*-mediated transformation, thus allowing the regeneration of transgenic plants at a higher rate (Heidmann et al., 2011; Lowe et al., 2016). This, in turn, has an important application in applied biotechnology for stable genetic transformation of recalcitrant species such as small grain cereals. Hence, *BBM* is one of the agriculturally important genes in crop research and trait improvement.

To date, no ortholog of the *BBM* gene has been characterized in a bread wheat. In this report, we describe identification of three homoeologs of the putative *BBM* gene mapped to chromosomes 6AL, 6BL and 6DL of the wheat variety Chinese Spring. Only two copies mapped to chromosomes 6AL and 6DL appear to be functional genes, whereas the B sub-genome copy is a pseudogene. The homoeologs demonstrate tissue-specific expression pattern and the transcripts are detected in the embryogenic microspore population in the wheat cultivar AC Andrew.

2. Materials and methods

2.1. Plant material and microspores isolation

Cultivation of the wheat plants were done as described in (Bilichak et al., 2015). Briefly, seeds of the wheat cultivar AC Andrew were germinated and grown in Cornell mix. Plants with established root mass were treated with 2.5 ml/l of Tilt™ (propiconazole, Syngenta) before the tillering and with Intercept™ (0.004 g/l of soil, Imidacloprid, Bayer) to control for pests. The tillers were harvested and kept at 4 °C for 3 weeks with their bases in distilled water and their heads wrapped in aluminum foil. Mid- to late uninucleate stage microspores were verified using a median floret and acetocarmine staining. Following cold pretreatment, the microspores were isolated from the spikes as described previously (Bilichak et al., 2015) with the following modifications. Up to fifteen spikes per extraction were surface sterilized with 1% sodium hypochlorite for 3 min followed by three washes with sterile distilled water. Florets were blended using Warring blender cup (VWR International) containing 50 ml of sterile extraction solution at 4 °C (Supplementary Table 1). The blended mixture was filtered and pelleted by centrifugation (100 × *g* for 5 min at 4 °C). The pellet was resuspended