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Research paper

Allergenicity of *Bet v 1* in wheat pollen after exposure to drought stress as a model for climate change

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Abstract
In the 21st century, climate changes and global warming have become critical concerns, in that they can affect many of the natural phenomena. It is reported that aeroallergen sensitization is influenced by changes in climate. Bet v 1 is an important birch pollen respiratory allergen and a prototype for the PR-10 protein family, which has been reported in wheat pollen. Two wheat genotypes were exposed to water deficit at the meiosis stage of anthers to investigate the effect of drought stress changes on wheat pollen *Bet v 1* expression. Then, mature anthers of wheat underwent molecular experiments in the anthesis stage. Expression analysis was carried out, using Real-time PCR, on the *Bet v 1* gene that encodes allergens. The results indicated the induction of *Bet v 1* in both genotypes in the water deficit condition. The genotype which had been improved by breeders for tolerating drought more than the other genotype revealed more increase in *Bet v 1* expression. Given the great spread of wheat, especially improved genotypes worldwide as a pivotal and crop plant and regarding wheat pollen effect on provoking allergy in humans, *Bet v 1* can cause new distress in human society. Therefore, this finding is considered as a new verification of concerns of climate change on human health, which emphasizes the importance of efforts to alleviate climate changes to avoid the risk of public health.

Keywords: Aeroallergen; Birch pollen allergy; Gene expression; Global warming

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Introduction

Extensive efforts have been made to study the global changes in the environment and their effects on biological events, agriculture, and the economy (Wayne *et al.* 2002). Studies on the climatic changes implied the quick modifications in recent 40 years, compared to last 2000 years, indicating the rate of average warming of the last 50 years to be almost two times bigger than the last 100 years (Ebi 2011). It has been claimed that climate changes will result in more frequent extreme climatic events (Hales *et al.* 2003). Drought is one of them, which has been consistent in many decades and has rapidly increased during recent decades in many regions around the world (Dai 2011).

Climatic changes and global warming, by increasing the risk of cardiovascular, infectious diseases, and most importantly allergies, can affect human health (Behrendt and Ring 2012). Their influence on health has been via affecting geographic ranges and intensifying the transmission of diseases and altering disease dissemination caused by aeroallergens (Ebi 2011). Moreover, the severity of diseases born out of pollen has been altered by the influences of climate changes on distribution, timing, allergenicity, and the number of pollen grains

(Storkey *et al.* 2014). Generally, it has been claimed that the interactions between impacts of extreme events like drought, people being exposed, and their sensibility, and preparations for overcoming the impacts are reasons of health risk of climate changes (Ebi and Bowen 2016).

Dissemination and outbreak of several diseases, especially those with sensitive vectors (like malaria (Caminade *et al.* 2014; Ryan *et al.* 2015), and dengue fever (Bouzid *et al.* 2014; Butterworth *et al.* 2017)), are affected by changes in the environment, in a way that the risk of their occurrence in unaffected areas increase. Asthma, allergic rhinitis (hay fever) (Ziska *et al.* 2011), and rhinosinusitis (Ebi 2011) are among three important allergic diseases caused or intensified by aeroallergens. The prevalence of asthma in Iran has been increasing in the last two decades (Ghaffari and Aarabi 2013). Some studies on allergic patients in Iran have shown the importance of weed and grass pollen more than any other studied aeroallergens (Fereidouni *et al.* 2009; Assarehzadegan *et al.* 2013).

Climatic changes have various effects on allergic diseases, ranging from longer pollen season, which causes allergic sensitization and enhanced period of allergy symptoms, increased pollen numbers (Ziska *et al.* 2011), changes in pollen dispersion, and increasing the expression of allergenic proteins (Todea *et al.* 2013). On the other hand, early flowering in moderate winters is caused by climatic changes (Anderson *et al.* 2012), which in turn make the duration of exposure to pollen grains longer (Ziska *et al.* 2011). Climate changes' impact on the flowering trends was studied in 141 species in North America and results indicated a mean of 2.4 days (-1 °C) of early flowering (Calinger *et al.* 2013). Changes in aeroallergen production timing, such as early flowering, occur in woody plants, grasses, and even mold spores (Cecchi *et al.* 2010). Thereafter, if warming causes long-time climate change, people will be exposed to seasonal allergens for a long time which, in turn, will lead to allergic diseases such as rhinitis and asthma (Cecchi *et al.* 2010; Ziska *et al.* 2011). All of these events might change the seasonality of pollen-borne diseases (Ebi 2011).

Enhancement of environmental carbon dioxide (CO_2) has also been demonstrated to be influential in increasing the amount and allergenicity of pollen of many taxa, especially in urban places (Ziello *et al.* 2012). Pine trees grown under high CO_2 produced pollen in younger ages with smaller sizes, which result in greater pollen production (LaDeau and Clark 2006). The concentration of tree pollens is also affected by high temperature, which also increases allergen sensitization among patients (Kim *et al.* 2011). However, anthropogenic rise in CO_2 levels has been claimed to be more important than temperature increase (Ziello *et al.* 2012).

Birch produces different allergen molecules, among which Bet v 1, Bet v 2, and Bet v 4 are the most prevalent ones (Ciprandi *et al.* 2016). Bet v 1, which is an important birch pollen respiratory allergen, is a prototype for the PR-10 (pathogenesis-related) protein family (Roth-Walter *et al.* 2014). Bet v 1 is the main allergen of silver birch (*Betula verrucosa*), which binds to IgE to induce birch pollen allergy (Kleine-Tebbe *et al.* 2017b). Studies showed that among the PR-

10 family, sensitivity to Bet v 1 was more common between the examined patients, followed by Cor a and Mal d 1 (Blankestijn *et al.* 2017). A younger group of people showed more sensitivity to Phl p 1 than others, and then group 2 mite allergens, Bet v 1, and Fel d 1 (Stemeseder *et al.* 2017). About 12%. 17%, and 25% of children at ages of 4, 8, and 16, respectively were found to be IgE reactive to Bet v 1, besides the fact that in hierarchic intrarelationship of allergens, Bet v 1 was the first allergen (Westman *et al.* 2015). A literature review on allergenicity showed that studying Bet v 1 is important in the diagnosis of allergy patients, providing consultation to them, and allergen immunotherapy (Kleine-Tebbe *et al.* 2017a).

Despite the importance of drought stress as an extreme event caused by climate change, the rarity in studies of drought stress on allergenicity is explicit. In a study on apple allergens, it was proved that water stress caused up-regulation of Mal d 1.04 and Mal d 4.01 (two apple allergens) in apples (Botton *et al.* 2008). However, other genetic reasons for the exacerbated prevalence of allergic diseases are not known and limited investigations have been done on the role of climatic changes on gene expression of pollens allergenicity of wheat. In the present study, we aimed to investigate the water deprivation effect on the expression level of *Bet v 1*, one of the most allergenic genes in bread wheat pollen.

Material and Methods

Plant material and water treatment

Two different wheat genotypes Dezfoul [D-10, Petheenr.2123/Bolani that was selected based on ARWYT-DROUGHT experiments and previous experiments (Mehri *et al.* 2020)], as a drought-resistant genotype, and Shiraz [Sh, Azd/3/“Ald”s//Gv/D630 (Seed and Plant Improvement Institute, 2015)] as a drought susceptible genotype, were investigated in a factorial experiment with two levels of irrigation (normal irrigation and water deficit) in a randomized complete block design with three replications over time; the sampling dates being the replicates. Plants were sown in pots of 20 cm height and 15 cm diameter that were filled with an equal volume of a 6:3:1 mixture of soil: sand: green manure. Irrigation treatment was based on the soil moisture characteristic curve, in a way that normal watering imposed 0.05 bars and stress treatment imposed 0.1 bars soil pressure head. Since in most instances in nature the drought stress of arid climate mostly occurs around the meiosis phase of pollens (Boyer and Westgate 2004), all of the pots were watered normally until two weeks before meiosis. For determining the meiosis stage, samples of two genotypes were sown in two pots two weeks before the main pots, and their meiosis stage was monitored regularly using the 1% acetocarmine squash method. As soon as meiosis was observed in these two pots, water deficit imposed in drought-stress treatments and normal watering was resumed after meiosis (Figure 1). Anthers were sampled at the time of meiosis and were stored in liquid nitrogen, then transferred to −80 °C freezer in the laboratory.

RNA extraction

Total RNA for reverse transcription was isolated from sampled pollens, using a RiboEx RNA extraction kit (GeneAll, Korea) according to the kit protocol. An amount of 50 ng of DNaseI-treated RNA was converted into the first-strand cDNA, using oligo(dT)$_{18}$ and HyperScript First Strand Synthesis Kit (GeneAll, Korea), utilizing iCycler Thermal Cycler (BioRad, USA) based on the kit protocol.

Quantitative reverse transcription polymerase chain reaction

Real-time PCR reactions were performed in final volumes of 20 μl, which contained 4 μl Hot FIREPol EvaGreen qPCR Mix Plus (no ROX) (Soils BioDyne, Estonia), 1 μl of each primer, 12 μl nuclease-free water, and 2 μl RT reaction product, using Rotor-Gene (Qiagen, Germany). The reactions were as follows: incubation at 95 ℃ for 15 min, followed by 40 cycles of 95 ℃ for 30 sec, an optimized annealing temperature of 54 ℃ for 45 sec, and 72 ℃ for 45 sec. *Bet v I* allergen [*Triticum aestivum*] (gi|63021412) specific primer set was designed via Primer Premier 5. The sequence of the designed primer set was as follows: 5’-CCCCGAGCAGTACAAGAG-3’ and 5’-ACCTTCTTCTCGTCGTCC-3’. It has been demonstrated that *GAPDH* housekeeping gene shows an average and acceptable stability compared to other housekeeping genes in pollen grains (Zareii 2010). Thus, real-time PCR results were normalized by *GAPDH* for each sample. Run melting curve was executed to validate the specificity of EvaGreen, and primer-dimer absence. The relative expression level of the *Bet v 1* gene was then calculated using the p-value of $\Delta\Delta CT \leq 0.05$ (Livak and Schmittgen 2001), and confidence interval of $\propto = 0.01$, normalized to the CT values of *GAPDH*. In the real-time PCR analysis, data for relative expression is based on a small sample size, hence, obtained data did not have a normal distribution. Therefore, the significant test was done by bootstrap resampling method with 1000 samples by REST (Pfaffl *et al.* 2002).

Results

It was found that drought stress caused a significant increase in the expression of *Bet v 1* in the Dezfoul cultivar (Figure 2). The expression of *Bet v 1* in Dezfoul under drought conditions was about 15 times higher than normal irrigation. In addition, the expression levels of the Shiraz cultivar also increased substantially in the drought condition (8.3 times) (Figure 2). Overall, *Bet v 1* showed higher expression in the Dezfoul genotype compared to Shiraz.

Discussion

Wheat (*Triticum aestivum*) is considered a vital food for many people around the world, which is cultivated in vast areas (about 216 million hectares) of croplands (FAO 2019). In Iran, 3.8 million hectares of arable lands are under rainfed cultivation (Iran Communication and Information Technology Organization 2016), which encounter several abiotic stresses, especially drought. Such a great amount of wheat planting areas has the potential to produce an ample volume of pollen. Wheat causes IgE-mediated allergy by different allergens (Pahr *et al.* 2012). However, some people show sensitization to food PR-10 proteins but not to pollen PR-10 (Blankestijn *et al.* 2017).

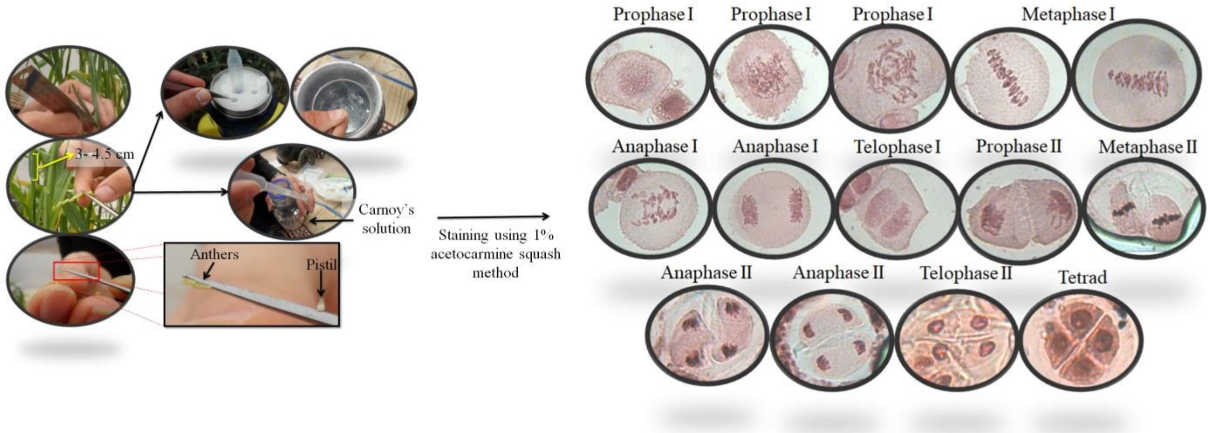

Figure 1. Sampling and anther staining. When the booting size was around 3 to 4.5 cm (about two weeks after irrigation was withheld in the water stress treatments), sampling was started. To ensure that the anthers were in the meiosis stage, half of the anthers of one spikelet were transferred to liquid nitrogen and the other half were transferred to Carnoy's solution to monitor the meiosis stage regularly using the 1% acetocarmine squash method.

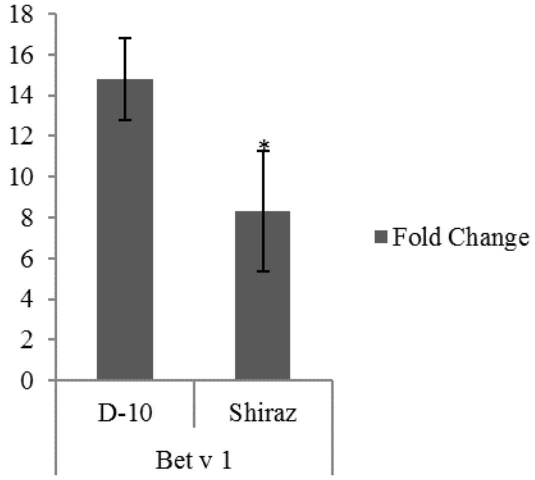

Figure 2. Differential expression of *Bet v 1* in Dezfoul (D-10) and Shiraz wheat genotypes under drought stress conditions compared to normal irrigation. Vertical bars indicate standard errors. Significance tests were carried out by the bootstrapping method.

Many studies on wheat allergens are limited to allergens that root in consuming wheat as a food (Palosuo *et al.* 1999; Sampson 2001) but not the allergies triggered by the wheat pollen.

It has been reported that exposure to environmental stresses could change the severity of pollen allergens' negative effects (Hamaoui-Laguel *et al.* 2015; Zhang *et al.* 2015; Höflich *et al.* 2016). Studies have shown that allergic diseases in humans are exacerbated by global warming and climate change. Most of the studies have focused on pollen concentration (Albertine *et al.* 2014; Lake *et al.* 2017), distribution (Lake *et al.* 2017), timing (Anderson *et al.* 2012), and length of pollen season (Anenberg *et al.* 2017). Some of these researches estimated a duplication in ragweed sensitization by 2041-2060 (Lake *et al.* 2017). It has been reported that global elevated CO_2 and drought stress affect pollen transcriptome and allergenic potential of ragweed pollen (El Kelish *et al.* 2014). Studying the total proteome of wheat pollen in the meiosis stage indicted an induction of Bet v 1 under drought stress (Fotovat *et al.* 2017). In the present study, the rising of *Bet v 1* expression in both studied genotypes under drought, indicates its sensibility to the water limitation period. On the other hand, Dezfoul, which is considered a drought-tolerant genotype, demonstrated a significantly greater increase in *Bet v 1* expression, compared to Shiraz (a very susceptible genotype to drought stress). Drought is the most crucial abiotic stress, and estimations have indicated its increasing trend and it will get more severe in oncoming years (Dai 2011). Plant breeders have made remarkable efforts to enhance wheat tolerance to drought, thereby, improving drought tolerance of wheat cultivars and their distribution in farms, which may raise the potential of their allergenicity. To our knowledge, this is the first time to prove that drought stress has brought about up-regulation of *Bet v 1* gene expression in the wheat pollen. Accordingly, the risk of wheat pollen-born allergies may follow a mounting drift in the world population in the next few years. Consequently, the results suggest that considering the drought and its effect on increasing pollen allergens, and the possibility of spreading more allergen cultivars, seeking ways to alleviate climate changes' influence on alleviating the symptoms of allergy in human society is essential.

Conclusion

Our results provide convincing evidence of a significant increase in *Bet v 1* expression, as an important allergen, in the wheat pollen under drought conditions. Taken together, in the light of widespread disperse of wheat worldwide, particularly genotypes that are released for their especial potential in tolerating stress, and regarding wheat pollen effect on provoking allergy in humans, Bet v 1 may pose extensive public health burden by affecting allergic disease frequency due to climate changes. Therefore, this finding emphasizes the importance of efforts to alleviate climate change to avoid the risk of public health.

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Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

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آلرژی زایی Bet v 1 در دانه گرده گندم در مواجهه با تنش خشکی، مدلی برای تغییرات اقلیمی

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چکیده

تغییرات اقلیمی و گرمایش جهانی از مشکلات اساسی قرن ۲۱ ام محسوب می‌شود، به نحوی که می‌تواند بسیاری از پدیده‌های طبیعی را تحت تأثیر قرار دهد. گزارش‌هایی وجود دارند مبنی بر این که حساسیت‌زایی آلرژن‌ها تحت تأثیر تغییرات اقلیمی قرار دارند. آلرژن Bet v 1، آلرژن تنفسی مهم دانه گرده درخت غان و پروتوتایپی از خانواده پروتئینی RP-10 است که در دانه گرده گندم گزارش شده است. جهت بررسی اثر تغییرات تنش خشکی روی بیان ژن Bet v 1 در دانه گرده گندم، دو ژنوتیپ گندم در مرحله میوز بساک تحت تأیر تنش خشکی قرار گرفتند. سپس بساک‌های بالغ گندم در مرحله گرده‌افشانی مورد آزمایش‌های مولکولی قرار گرفتند. تجزیه بیان ژن Bet v 1 که کدکننده آلرژن است، با استفاده از دستگاه Real-time PCR انجام گرفت. نتایج نشان‌دهنده القای Real-time PCR در هر دو ژنوتیپ بود. ژنوتیپی که توسط اصلاح‌کنندگان برای تحمل گرما اصلاح شده بود، دارای افزایش بیان بیشتر ژن Bet v 1 بود. به دلیل گسترش گندم، به ویژه گندم‌های اصلاح شده، به عنوان گیاه اساسی و با در نظر گرفتن اثر دانه گرده گندم روی افزایش آلرژی در انسان، ژن Bet v 1 می‌تواند مشکل جدیدی در جامعه بشریت باشد. بنابراین، این یافته تأیید جدیدی است از نگرانی در مورد اثر تغییرات اقلیمی روی سلامت انسان که بر اهمیت تلاش برای کاهش تغییرات اقلیمی جهت پرهیز از به مخاطره انداختن سلامت عمومی تأکید دارد.

واژه‌های کلیدی: آلرژن تنفسی؛ آلرژی دانه گرده درخت غان؛ بیان ژن؛ گرمایش جهانی