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CHARACTERIZATION AND MAPPING OF A WILD BARLEY *EIBI1* MUTATION OF A GENE ESSENTIAL FOR LEAF WATER CONSERVATION

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Abstract

While testing drought resistance of wild barley (*Hordeum spontaneum*) genotypes from Wadi Qilt population in Israel (longitude 35.38, latitude 31.83, altitude 50 m, annual temperature 24.8 °C, annual rainfall 144 mm, annual evaporation 3300 mm), we discovered a spontaneous mutant, hypersensitive to drought and readily exhibiting a wilty phenotype under water deficit. This spontaneous wilty mutant was investigated for exogenous ABA response, stomatal movement, endogenous ABA content, cuticle permeability, and the relationship of transpiration with stomatal density and cuticle permeability. An approximately 3:1 segregation ratio of wild type to mutant among 158 F_2 plants derived from a cross of *eibi1* by Morex revealed that the *eibi1* high water loss rate was caused by a single recessive nuclear mutation. This mutation was mapped on chromosome 3H short arm between simple sequence repeat markers (SSR) Bmag0603 and Bmac0067, and co-segregated with Bmac0828.

Results and discussions

A. *eibi1* is a recessive nuclear mutation

The typical features of this mutant were: a. sensitivity to drought stress; b. fast water loss of detached leaves; c. leaf twisting, d. leaf dark green; e. shorter stature; f. twisted peduncle; g. producing significantly more tillers; h. reduced fertility; i. decreased spike size (**Fig. 1**).

This spontaneous mutant was named ***eibi1***. Segregation analysis of the F2 progenies of two crosses: Morex (a cultivar) × *eibi1* and 23-19 (a near-isogenic line of *eibi1*) × *eibi1* showed an approximately 3:1 segregation ratio of wild type to mutant, indicating that the *eibi1* phenotype was caused by a single recessive nuclear mutation.

Fig. 1. Comparison of *eibi1* and its isogenic wild type

B. *eibi1* is hypersensitive to drought stress

The detached leaves of *eibi1* plants lost 43.2% of the initial weight within one-hour of dehydration while the wild type detached leaves lost only 4.6%. In the same experiment, the detached leaves of tomato *flacca* (a typical wilty mutant) lost significantly less water than *eibi1* within one-hour of dehydration whereas its near-isogenic line RR (*Lycopersicon esculentum* Mill. cv. Rheinlands Ruhm) lost significantly more water than the wild type of *eibi1* (**Fig. 2**).

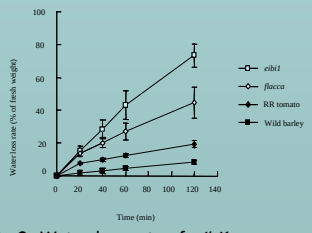

Fig.2. Water loss rate of *eibi1*

C. *eibi1* is defective in cuticle

Chlorophyll efflux rate of leaves was greater in *eibi1* than in wild type (**Fig.3**). All these lines of evidence suggest that *eibi1* is defective in cuticle, an important interface between the plant and the surrounding atmosphere.

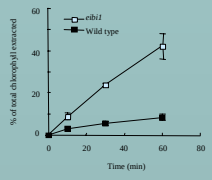

Fig.3. Chlorophyll efflux of *eibi1*

E. Genetic mapping of the *eibi1* mutation

The ***eibi1*** was mapped on chromosome 3H short arm (**Fig. 6**).

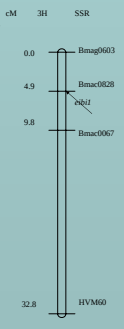

Fig. 6. Genetic mapping of *eibi1*.

D. *eibi1* is not attributed to ABA deficiency and insensitivity

Wild-type and *eibi1* shoots had the same ABA contents under control conditions, whereas drought stress increased ABA contents in both *eibi1* and wild type to the same level (**Fig. 4**), indicating that *eibi1* had the normal ABA content and the ability to accumulate ABA under drought stress. Therefore, the drought supersensitiy of *eibi1* is not attributed to ABA deficiency. The stomatal movement of *eibi1* was normal, but *eibi1* had more than 10 times higher transpiration rate than wild type in the dark (**Fig.5**).

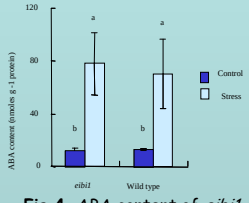

Fig.4. ABA content of *eibi1*

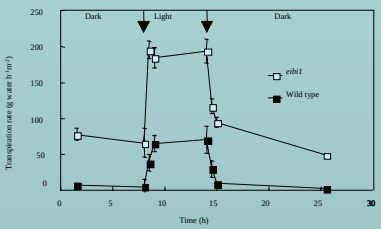

Fig.5. Transpiration rate of *eibi1*

Conclusions

-A novel gene, *eibi1*, was discovered. It functions in the transport-limited layer in cuticle and is essential for plant drought resistance.

- The *eibi1* mutation was located on chromosome 3HS.

- The mutant may be used as an important tool to unravel one of the genetic pathways of leaf water conservation.

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