Evaluation of susceptibility of 12 Walnut genotypes to sudden cold of 2008 and frost injury

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Frequent frosts occurs during winter and spring in Khorasan (Iran), that causes serious damage to walnut, which necessitating the selection of resistant cultivars. Before introducing new genotype, their capacity for adaptation to different condition needs to be studied. Walnut production in Khorasan has been increasing through area expansion and new genotypes. In this area affective freezes seldom damages walnut trees. The minimum temperature on winter of 2008 was about -22^0C. The objective of this study was to evaluate cold hardiness in 12 various walnut genotypes (K21B5/13, K21B5/15, K21B6/17, K21B6/18, K28B2/5, K28B2/6, K23B5/13, K23B5/14, K21B3/8, K21B9/25, K28B3/8, K21B1/3) which planted in 1986 on seedling at the genotype collection of Khorasan Agricultural and Natural Sources Researches Center. The results on 12 walnut genotypes showed that K21B5/13, K21B5/15, K21B6/18, K21B3/8, and K28B3/8 genotypes damaged more than other genotypes, and K23B5/13, K23B5/14 and especially K21B9/25 genotypes, showed to be resistance to minus 22°C. Results showed straight correlation between ionic leakage and visual observation. Minimum ionic leakage was observed in K21B9/25 genotype and visual observation verified its cold hardiness. Flower buds in K21B5/15 genotype and vegetative buds in K21B3/8 genotype were damaged completely. Sexual buds were the most sensitive organs of tree. Annual shoot was damaged more than biennial shoot in trees.

Introduction

In northern latitudes of hemisphere, low temperature is a major environmental factor limiting the productivity of perennial crops. Low temperature is a dominant factor in the distribution, growth, and survival of woody plants (2, 3). Sudden exposure of unhardened plants to freezing temperatures typically results in injury to their shoots, cambium, and roots and often leads to death of the plants. Cold hardiness varies greatly among species, genotypes, and even different parts of the same plant (4). Skrøppa (1991) found differences in frost hardiness between two populations of *Picea abies* that originated only 60 kilometers apart at the same altitude and latitude. In general, reproductive structures, roots, and young leaves are particularly sensitive to low temperature. Several methods have been proposed for selecting frost tolerant genotypes in stone fruit species, such as visual observations (7) and ionic leakage (5). The subject is confined to aerial parts of walnut trees. Hence, the major climatic factor associated with walnut frost injury in northeast Iran is the severity of winter, i.e. low temperatures during January, February, and March.

Materials and methods

Experiments were carried out using 12 walnut genotypes (K21B5/13, K21B5/15, K21B6/17, K21B6/18, K28B2/5, K28B2/6, K23B5/13, K23B5/14, K21B3/8, K21B9/25, K28B3/8, K21B1/3) that grafted in 1986 on seedling at the genotype collection (36°17′N and 59°36′E and altitude 1012m) of Khorasan Agricultural Researches and Natural Sources Center. Buds per genotype were dissected and the rate of injury determined visually and relative ionic leakage. Also, one and two year old shoots ca. 30 cm long were randomly collected on 1 Feb. of 2008 from mature trees and the rate of injury determined ionic leakage. Electrolytic conductivity (C1) was measured after 24 h shake. Solutions and samples were then autoclaved to kill the tissues and conductance was again measured (C2). Ionic leakage (C %) was expressed as the percentage of the final reading (C% = C1/C2 × 100). The categories recorded were as follows: uninjured, partially injured (all of the primordia in a flower bud were brown) and injured (those which had brown pistils and or stamens) as shown by Szabó et al. (1996). The main objective of this research was to evaluate and identification of cold hardiness on 12 walnut different genotype with a special emphasis on relative ionic leakage and visual observation for measuring hardiness characteristics.

Results and discussion

The minimum temperature on winter of 2008 was about -22°C. The chilling-injured buds were characterized by both relative ionic leakage and visual observation compared to non-injured samples. The number of male and female flower buds containing brown primordia was high in genotype of K21B5/15. The damaged female flower buds were determined by visual observations (Table 1). Maximum percentage of uninjured flower buds was 80% in female flower buds. Studies on relative ionic leakage of female flower buds showed that relative ionic leakage of K21B9/25, K23B5/14 and K23B5/13 genotype were significantly lower than other genotypes. Highest relative ionic leakage was observed in K21B5/21 genotype (Fig1). In male flower buds, the lowest relative ionic leakage were observed in K21B9/25, K23B5/13 and K23B5/14 genotypes and the highest ionic leakage were observed in K21B5/15 and K21B5/13 genotypes. Significant difference was observed in ionic leakage of male flowers (Fig1.). In visual studies maximum percentage of injured buds was measured on K21B5/15 and K21B5/13 genotypes and minimum percentage at K23B5/13, K23B5/14, K28B2/5 and K21B9/25. In K21B9/25, K23B5/13 and K23B5/14 genotypes, ionic leakages were same and significantly lower than other genotypes. Other genotypes were same and no significant difference was observed. In visual study maximum percentage of injured buds was measured on K21B5/15, K21B6/18 and K21B3/8 (Table 1).

Table1. Visual observations results of female and male flower and vegetative buds of 12 walnut genotypes

Genotype codes	Visual damaged percentage of female flower buds			Visual damaged percentage of male flower buds			Visual damaged percentage of vegetative buds		
	uninjured	Partially injured	injured	uninjured	Partially injured	injured	uninjured	Partially injured	injured
K21B5/13	10	10	80	10	30	60	40	20	40
K21B5/15	0	20	80	10	20	70	20	20	60
K21B6/17	20	40	40	20	30	50	20	60	20
K21B6/18	20	40	40	20	30	50	30	50	40
K28B2/5	40	40	20	60	40	0	80	20	0
K28B2/6	40	40	20	60	30	10	80	20	0
K23B5/13	80	20	0	90	10	0	100	0	0
K23B5/14	80	20	0	90	10	0	100	0	0
K21B3/8	20	40	40	10	40	50	20	40	40
K21B9/25	80	20	0	90	10	0	100	0	0
K28B3/8	20	40	40	20	40	40	50	30	10
K21B1/3	20	40	40	10	60	30	0	100	0

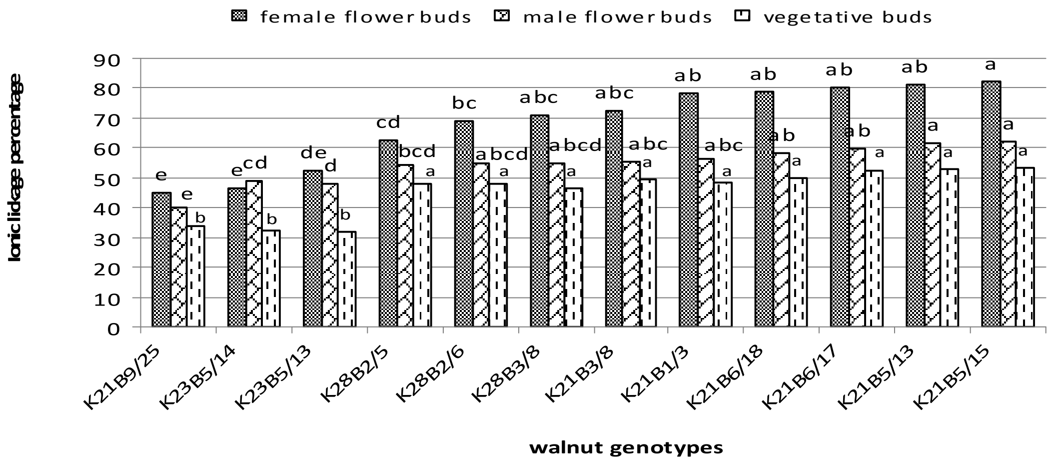

Fig1. Relative ionic leakage of female and male flower buds and vegetative buds of 12 walnut genotypes

Maximum and minimum ionic leakage of annual shoots was observed in K21B5/15 and K23B5/13 respectively. In spite of annual shoots showed higher resistance than reproductive and vegetative buds, but they damaged too, such as K21B5/15 genotype had high relative ionic leakage (Fig.2). Maximum and minimum ionic leakage was observed in K21B5/15 and K23B5/13 respectively. The lowest ionic leakage was observed in biennial shoots.

The freezing tolerance of woody plants, as measured by ionic leakage after a freeze-thaw event, is influenced by the freezing temperature, cultural environment before test, cultivar, and test time (Eugénia et al 2003). Comparison of relative ionic leakage in various genotypes and in various parts of the walnut trees indicates that the most sensitive part of plant shoots are female buds in the next order male buds, vagetative buds, annual shoot and finally biannual shoots.

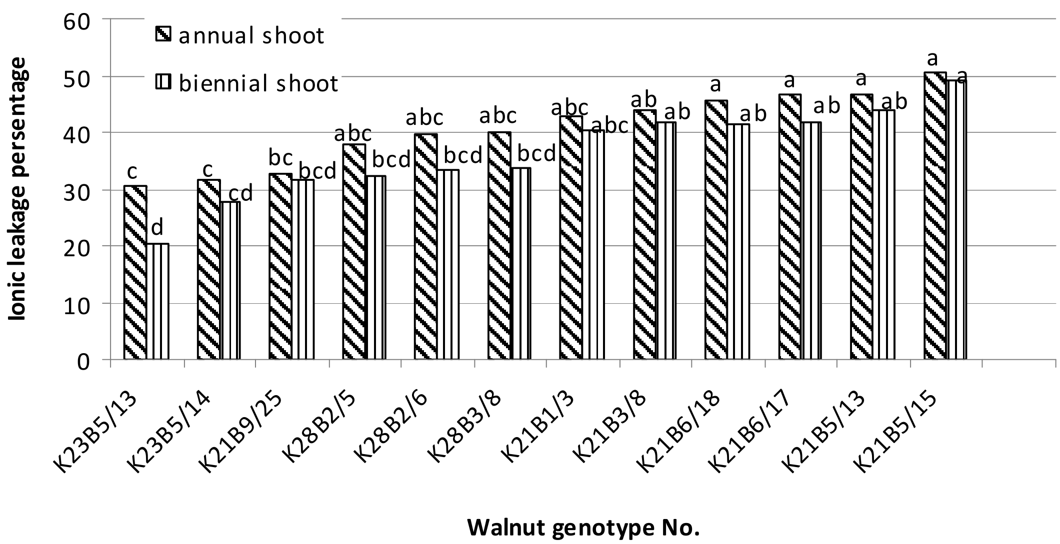

Fig2. Relative ionic leakage of annual and biennial of 12 walnut genotypes

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