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Original article

Nuclear DNA contents of *Echinchloa crus-galli* and its Gaussian relationships with environments

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A B S T R A C T

Previous studies on plant nuclear DNA content variation and its relationships with environmental gradients produced conflicting results. We speculated that the relationships between nuclear DNA content of a widely-distributed species and its environmental gradients might be non-linear if it was sampled in a large geographical gradient. *Echinochloa crus-galli* (L.) P. Beauv. is a worldwide species, but without documents on its intraspecific variation of nuclear DNA content. Our objectives are: 1) to detect intraspecific variation scope of *E. crus-galli* in its nuclear DNA content, and 2) to testify whether nuclear DNA content of the species changes with environmental gradients following Gaussian models if its populations were sampled in a large geographical gradient. We collected seeds of 36 Chinese populations of E. crus-galli across a wide geographical gradient, and sowed them in a homogeneous field to get their offspring to determine their nuclear DNA content. We analyzed the relationships of nuclear DNA content of these populations with latitude, longitude, and nineteen bioclimatic variables by using Gaussian and linear models. (1) Nuclear DNA content varied from 2.113 to 2.410 pg among 36 Chinese populations of *E. crus-galli*, with a mean value of 2.256 pg. (2) Gaussian correlations of nuclear DNA content (y) with geographical gradients were detected, with latitude (x) following $y = 2.2923 * e^{\frac{-(x-24.9360)^2}{2*63.7945^2}}$ (r = 0.546, $P < 0.001$), and with longitude (x) following $y = 2.2933 * e^{\frac{-(x-116.1801)^2}{2*44.7450^2}}$ (r = 0.672, $P < 0.001$). (3) Among the nineteen bioclimatic variables, except temperature isothermality, precipitations of the wettest month, the wettest quarter and the warmest quarter, the others could be better fit with nuclear DNA content by using Gaussian models than by linear models. There exists intra-specific variation among 36 Chinese populations of *E. crus-galli*, Gaussian models could be applied to fit the correlations of its Nuclear DNA content with geographical and most bioclimatic gradients.

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1. Introduction

Nuclear DNA content is significant in many biological fields. There is a continuing need to obtain more Nuclear DNA content data for plant taxa (Bennett, 1998; Soltis et al., 2003; Walker et al., 2006). Many of early published nuclear DNA content data were based on single sample and hence favor the idea that nuclear DNA content is constant for a species. Since 1980's, intraspecific variation has been reported for a range of unrelated species (Laurie and Bennett, 1985; Bennett, 1998; Reeves et al., 1998). Bennett (1985) reported intraspecific variation of nuclear DNA content in 24 gymnosperms, their variation being from 4% to 288%. Achigan-Dako et al. (2008) measured DNA 2C-values of 366 individuals from 117 populations of *Lagenaria siceraria* from Africa, America and Asia, and found that 2C-value in *L. siceraria* varied from 0.683 pg to 0.776 pg, a 12% variation among all populations. The variation of nuclear DNA content among different populations was also reported from *Aegilops squarrosa* (Furuta et al., 1975), *Dactylis glomerata* (Reeves et al., 1998) and *Sesleria albicans* (Lysâk et al., 2000).

However, the intraspecific variations in nuclear DNA content have been contradictory reported. Despite many reports of such variations in literature, others have failed to substantiate them. For example, no obvious variation of nuclear DNA content among

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different varieties was reported for *Cajanus cajan* (Oreilhuber and Obermayer, 1998) and *Triticum aestivum* (Wetzel et al., 1999). Greilhuber and Ebert (1994) found differences only up to 1.054-fold in single experiments, while Laurie and Bennett (1985) reported a variation up to 37% in cultivated maize. Therefore, there is a need for careful detailed systematic checking, using widely-distributed materials with clear taxonomic circumscription, to test the universalism of intraspecific variation in nuclear DNA content.

Close relationships of plant nuclear DNA content with environmental factors have been reported. However, some studies on the variation of nuclear DNA content across geographical and climatic gradients even produced conflicting results (Reeves et al., 1998; Knight and Ackerly, 2002). On latitude gradient, early studies indicated a positive correlation of nuclear DNA content of a species with its latitude (Levin and Funderburg, 1979; Freshwater, 1988). In contrast, Grime and Mowforth (1982) found a negative correlation between nuclear DNA content and latitude for 169 species in the British flora (Rayburn et al., 1985). Other studies have found no relationship (Teoh and Rees, 1976; Creber et al., 1994). With altitude gradient, positive (Rayburn and Auger, 1990), negative (Creber et al., 1994; Reeves et al., 1998) and non relationships (Palomino and Sousa, 2000) of nuclear DNA content in different species have also been reported. Reeves et al. (1998) found a decline in C-value with increasing altitude in *Dactylis glomerata* natural populations. Razafinarivo et al. (2012) found that nuclear DNA content of coffee trees increased following a north to southeast gradient in Madagascar and an east to west gradient in Africa. Bottini et al. (2000) found that diploid species of *Berberis* with lower nuclear DNA content grow in high-elevation sites having greater rainfall but lower water availability, while those with higher nuclear DNA content are associated with half-elevation forests where the vegetative period is longer, the water availability is greater and the temperatures are higher. It should be pointed that either positive or negative correlation of nuclear DNA content with latitude (or altitude) was reported as linear in most early documents.

As relevant works go further, non-linear relationships of nuclear DNA content with environmental gradients have been reported. Rayburn and Auger (1990) found that for *Zea mays* maximum 2C-values occurred at intermediate elevations with a trend toward reduced 2C-values at both high and low elevations. By using quantile regression, Knight and Ackerly (2002) found that species with large nuclear DNA content in the ecologically diverse California flora occur at intermediate July maximum temperatures, and decline in frequency at both extremes of the July temperature gradient. According to Bennett et al. (1982), Bennett (1987) and Rayburn and Auger (1990), species with greater nuclear DNA content may be more frequent in the temperate mid-latitudes and at intermediate elevations, with a trend towards species with reduced nuclear DNA content at the equator and the poles and at high and low elevations (Knight and Ackerly, 2002). *Silene ciliata* is a small perennial inhabiting the mountain ranges of the European Mediterranean basin. Garcia-Fernandez et al. (2012) found significant differences in DNA C-value among their studied three populations. The largest DNA C-value found at the intermediate population may be associated to lower environmental stress at the mid elevation. Therefore, the relationship between nuclear DNA content and geographical gradients may not be linear, but non-linear. Gaussian curve models may be suitable for analyzing the relationship of nuclear DNA content with environmental variables, which needs evidences to confirm.

Climatic factors covarying with geographical gradients may represent selection pressures on nuclear DNA content, yet they have been tested in only a few taxa. For example, Wakamiya et al. (1993) reported a positive correlation of nuclear DNA content with local annual precipitation and a negative correlation with mean maximum temperature, Price et al. (1981) detected a positive correlation between annual precipitation and plant nuclear DNA content, and MacGilivray and Grime (1995) found that frost-resistant species tended to have greater nuclear DNA content. Wakamiya et al. (1993) reported negative correlations of nuclear DNA content with the lowest average annual and monthly precipitation, the highest average monthly spring air temperature of the habitats in *Pinus* species.

Numerous studies have been conducted on the mechanisms about the relationship between nuclear DNA content and environment variables (Grime and Mowforth, 1982; Grime et al., 1985; Bennett, 1987; Mowforth and Grime, 1989; MacGilivray and Grime, 1995; Grime, 1998). Nuclear DNA content correlates closely with many cellular and some important morphological characters (Bennett, 1998), including seed-bearing age, and seed dimension (Wakamiya et al., 1993). A positive relationship of nuclear DNA content with seed size has been reported in Soybean strains (Chung et al., 1998), British plants (Thompson, 1990; Grime, 1998), *Crepis* (Jones and Brown, 1976), *Allium* and *Vicia* (Bennett, 1972), Poaceae and Fabaceae species (Mowforth, 1985). There existed a positive correlation between the nuclear DNA content and 1000-seed mass of the plants whose seeds were dispersed by animal-eating and water, which provides references to reveal the mechanisms of plant seed dispersal, distribution, and genome evolution (Bai et al., 2013).

Echinochloa crus-galli (L.) P. Beauv. is a worldwide distributed weedy species. In China the species has been reported from tropical to cool temperate areas (Chen et al., 2006). There is no document about its intraspecific variation in nuclear DNA content. Therefore, *E. crus-galli* is an ideal species for us to study nuclear DNA content intraspecific variation and its relationships with environmental gradients. The objectives of this paper are: 1) to detect variation scope of *E. crus-galli* in nuclear DNA content, 2) to testify whether the nuclear DNA content of *E. crus-galli* changes with geographical and climatic factors following Gaussian models if its populations were sampled in a large latitude gradient.

2. Materials and methods

2.1. Materials

The localities of 36 populations are presented in Table 1, which were collected from eastern part of China and Hainan island of China (Fig. 1). The samples were confirmed as *E. crus-galli* according to the key for identifying Chinese species of the genus *Echinochloa* in "Flora of China" (Chen et al., 2006). The vouchers were deposited in the Herbaria of Shanghai Normal University. The seeds of the 36 populations were obtained from paddy fields of 12 provinces (Table 1) from May to October 2012, respectively. On 4 Jun 2013, 100 seeds of each population were selected to sow in a homogeneous paddy field, respectively.

Before sowing, the seeds were firstly soaked in tap water for 48 h, and then kept in an incubator at 35 °C for another 48 h for germination. The germinated seeds from the same population were sown in a plot (1 m × 1.2 m) in the homogeneous field. In each plot, 20 seedlings in one-leaved stage were kept and arranged 20–25 cm × 20–25 cm apart. Plots are kept 30 cm apart. During the cultivating period, the plots were not fertilized and other plants were weeded by hand.

The homogeneous field is located in Fuyang Experimental Station of China National Rice Research Institute (Hangzhou, Zhejiang Province, N29°44′45″ - 30°11′45″, E119°25′22″ - 120°9′18″), on the northern edge of the subtropical monsoon zone, with clearly four seasons, an annual mean temperature of 16.4 °C, an frost-free period of 231 days, an accumulated temperature (≥10 °C) of 4700 °C for about 225 days.

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