Saudi Journal of Biological Sciences (2015) **22**, 340–350

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers

Megahed H. Ammar, Salem S. Alghamdi, Hussein M. Migdadi *, Muhammad A. Khan, Ehab H. El-Harty, Sulieman A. Al-Faifi

Legume Research Group, Plant Production Department, Faculty of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

Received 28 October 2014; revised 6 February 2015; accepted 7 February 2015
Available online 16 February 2015

KEYWORDS

Faba bean;
Molecular diversity;
SRAP;
AFLP

Abstract Forty faba bean (*Vicia faba* L.) genotypes were evaluated for their agro-morphological performance and molecular diversity under Central Region of Saudi Arabia conditions during 2010–11 and 2011–12 seasons. Field performance results showed that faba genotypes exhibited a significant amount of variation for their agro-morphological studied parameters. Giza40 recorded the tallest genotype (139.5 cm), highest number of seeds per plants (100.8), and the highest seed yield per plant (70.8 g). The best performing genotypes were Giza40, FLIP03-014FB, Gazira1 and Goff1. Genetic variability among genotypes was determined using Sequence Related Amplified Polymorphism (SRAP) and Amplified Fragment Length Polymorphism (AFLP) markers. A total of 183 amplified fragments (alleles) and 1758 polymorphic fragments (bands) in SRAP and 202 alleles and 716 bands in AFLP were obtained using six SRAP and four AFLP primer combinations respectively. Polymorphism information content (PIC) values for AFLP and SRAP markers were higher than 0.8, indicating the existence of a considerable amount of genetic diversity among faba tested genotypes. The UPGMA based clustering of faba genotypes was largely based on origin and/or genetic background. Result of cluster analysis based on SRAP showed weak and not significant correlation while, it was highly significant based on AFLP analysis with agro-morphological characters ($r = 0.01$, $p > 0.54$ and $r = 0.26$, $p < 0.004$ respectively). Combined

* Corresponding author.
E-mail address: hmigdadi@ksu.edu.sa (H.M. Migdadi).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.02.005

SRAP and AFLP markers proved to be significantly useful for genetic diversity assessment at molecular level. They exhibited high discrimination power, and were able to distinguish the faba bean genotypes with high efficiency and accuracy levels.

1. Introduction

Faba bean (*Vicia faba* L.) is one of the most important legumes for its seed high protein content and nutritional value (Crepona et al., 2010). The crop is widely cultivated for use in both human food and animals feed. The world production of faba beans reaches 4.3 Million tons from total cultivated area of 2.55 Million hectares (FAOSTAT, 2012). Faba bean seeds contain relatively high proteins, carbohydrates, vitamins B, antioxidants and minerals. Protein content in different varieties varies from 26% to 41% (Picard, 1977). Carbohydrate contents varies from 51% to 68%, of which major proportion is contributed by starch (41–53%) (Cerning et al., 1975). Common bean exhibits significant antioxidant activities such as flavonoids, polyphenols and phenolics. The antioxidant properties of phenolic compounds may provide excellent dietary source for natural antioxidant for chronic disease prevention and health promotion (Oomah et al., 2006).

Genetic variation among faba bean genotypes is imperative for their efficient utilization in plant breeding schemes and effective conservation. Though morphological and agronomic traits are routinely used to access genetic diversity, they are not enough in numbers to cover the genome, are affected by environmental factors and developmental stage dependent. Development of molecular markers provided reliable information for evaluating and assessing the genetic diversity of *V. faba* germplasm; RAPD (Link et al., 1995; Alghamdi, 2008; Yassine et al., 2014), ISSR (Terzopoulos and Bebeli, 2008; Alghamdi et al., 2011), AFLP (Zeid et al., 2003) SSR (Pozarkova et al., 2002; Gong et al., 2010, 2011; Ma et al., 2011; Kaur et al., 2012; Yang et al., 2012; Yassine et al., 2014), and SRAP (Alghamdi et al., 2012). Molecular markers are sufficient in numbers, not influenced by environmental factors or by development stages (Bebeli and Kaltsikes, 1993).

Sequence-Related Amplified Polymorphism (SRAP) is a simple and efficient molecular marker technique with reasonable throughput rate, ability to disclose numerous co-dominant markers, more reproducible than RAPDs and are easier to assay than AFLPs and, most importantly, targeting of open reading frames (ORFs)(Li and Quiros, 2001). It was used in assessing genetic diversity in legumes including lentil (Rana et al., 2009), pea (Esposito et al., 2007) and Alfalfa (Vandemark et al., 2006; Ariss and Vandemark, 2007; Castonguay et al., 2010; Al-Faifi et al., 2013). It has been used for genetic diversity and phylogenetic studies in faba bean (Alghamdi et al., 2012).

Amplified Fragment Length Polymorphism (AFLP) is considered ideal marker system for DNA fingerprinting and diversity assessment (Vos et al., 1995). It was used for detection of genetic diversity among faba bean accessions (Zeid et al., 2003). Eight selected AFLP primer combinations produced 477 polymorphic fragments among 79 inbred faba lines. In another study, a sample of 39 spring type faba bean landraces from four provinces in China, were compared in diversity with 136 spring accessions from the rest of the world, including Africa, Canada, Asia, Europe, and 41 breeding lines from ICARDA (Zong et al., 2010).

This study aimed to evaluate field performance of forty faba bean genotypes, using agro-morphological traits and to assess levels of genetic diversity at molecular level using AFLP and SRAP molecular markers.

2. Materials and methods

Forty genotypes of faba bean were selected for this study. They included 33 accessions introduced from ICARDA and 7 local and exotic faba genotypes grown and adapted to Saudi ecosystem. The pedigree and origin of the selected faba bean genotypes are presented in Table 1. These accessions were grown at Dirab Experiments and Agricultural Research Station, South Riyadh (24° 43′ 34″ N, 46° 37′ 15″ E) in RCBD with three replications for two seasons 2010–11 and 2011–12. Seeds of faba bean genotypes were planted on 20th of October for the first season and on 1st of November for the second season. Monthly maximum and minimum temperature were recorded during the growing month (Supplementary Table 1). Seeds of faba bean genotypes were planted in two rows 3 m long with 50 cm distance between rows and 20 cm apart (3.0 m^2). Soil analysis classified soil as loamy sand with 0.3% organic matter and N% of 13.1, 20.6 ppm of absorbable P and 86.6 ppm of absorbable K. All cultural practices were applied as recommended, Diammonium phosphate (18%N_2, 46%P_2O_5) was added at the rate of 300 kg/ha during seed bed preparation. Seed inoculated by coating seed with Rhizobium (*Rhizobium leguminosarum*, *Vicia* ICARDA-441) before sowing was provided from Agriculture Research Centre/Giza, Egypt. Plots were immediately irrigated after sowing and then subsequently irrigated according to reading of class "A" pan 50% evaporation rate. Data on days to 50% flowering and 95% of maturing were taken by visual observation. At maturity, 10 randomly selected plants were used to measure agro-morphological traits i.e. plant height was measured from soil surface to the upper most tip of the plant, number of pods bearing branches/plant, number of pods/plant, number of seeds/plant and seed yield/plant.

Statistical analysis was performed for each season separately and after confirmation of errors compatibility for the two seasons, combined analysis was applied according to standard analysis of variance technique for RCBD design using *MSTATC* computer software and means were separated using Fisher's protected least significance difference (LSD) test at 0.05 level of probability (Steel and Torrie, 1980).

For molecular characterization, two-week old faba bean leaves from 40 selected genotypes were collected, dropped in