Scientia Horticulturae 200 (2016) 1–6

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Comparative proteomic analyses provide novel insights into the effects of grafting wound and hetero-grafting *per se* on bottle gourd

Lingping Wang[a], Guojing Li[a,b], Xiaohua Wu[a], Pei Xu[a,b,*]

[a] Institute of Vegetables, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[b] State Key Lab Breeding Base for Sustainable Control of Plant Pest and Disease, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

ARTICLE INFO

Article history:
Received 11 October 2015
Received in revised form
29 December 2015
Accepted 31 December 2015
Available online 11 January 2016

Keywords:
Bottle gourd
Hetero-graft
iTRAQ
Proteome
Wound stress

ABSTRACT

Grafting is a technology widely used in agriculture and biological research. During a hetero-grafting process, cutting wound and hetero-grafting *per se* are the two main stimuli invoking cellular and whole-plant responses. Despite the importance of dissecting the individual effects of the two in adequately interpreting experimental data, no currently published data are dedicated to comparing the effects of hetero-grafting *per se* and grafting wound. In this study, isobaric tags for relative and absolute quantitation (iTRAQ) technique-based proteomic analyses were conducted between various scion-rootstock combinations of bottle gourd. Our results demonstrated that effects of grafting wound and hetero-grafting *per se* were readily distinguishable at the proteome level. Grafting wound affected the proteomes differentially at the graft union and its upper vicinity. Its effects were also likely related to the stress resistance level of each genotype. The effects of hetero-grafting *per se* on proteome were markedly different between graft union and the upper vicinity. This study provides an explicit evidence for the effectiveness of the commonly used methods of eliminating the effects of grafting wound in a hetero-grafting system by setting up a homo-grafted control. The role of hydrogen peroxide as a signal molecule in hetero-grafting systems is suggested.

1. Introduction

Grafting is a commonly applied technology in vegetable and woody fruit plant production, which has the advantage of conferring the upper part of the graft (known as the scion) improved vigor, productivity and enhanced resistance to various biotic and abiotic stresses (Flores et al., 2010; Schwarz et al., 2010; Huang et al., 2010). Grafting is also widely used as a means to investigate cell-to-cell communication and long-distance signaling between the rootstock and the scion (Kassaw and Frugoli, 2012; Corbesier et al., 2007; Notaguchi et al., 2008; Pina and Errea, 2005; Pina et al., 2012). Technically, grafting is a two-step process comprising incision of the scion/rootstock and reunion of the two at the graft junction. A successful graft is marked by the complete fusion of the graft junction and reestablishment of vascular continuity (Fuentes et al., 2014; Goldschmidt, 2014). Depending on the compatibility between the rootstock and the scion, grafting can be made intra-specific (rootstock and scion belonging to the same botanical species), inter-specific (rootstock and scion belonging to different species of the same genus) or even inter-generic (Mudge et al., 2009).

Rootstock is known to play a critical role in conferring graft advantage. Cookson and Ollat (2013) reported that hetero-grafting, but not homo-grafting, had a major effect on expression of shoot apex genes. Rootstock-derived regulatory RNAs transmission to the scion is considered an important mechanism conferring disease resistance (Ali et al., 2013). Following hetero-grafting with a rootstock, various aspects of plant behavior such as water absorption, nutrient uptake, hormone metabolism and protective enzyme activity may be altered (Liu et al., 2014). However, ascribing such physiological or molecular changes to the effect of hetero-grafting *per se* needs caution, as grafting is always accompanied with cutting wound, an abiotic stress known to trigger multiple cellular responses easily confounding the effects of grafting *per se* (Irisarri et al., 2015; Clemente Moreno et al., 2014; Fluhr., 2001; Turnbull et al., 2002; Schilmiller and Howe, 2005; Stegemann and Bock, 2009). A thorough solution to the confounding effects of wounding was to monitor the cellular events occurring at early stages of graft union development using novel in vitro systems such as micro-callus suspension cultures (Prinsi et al., 2015; Moore and Walker, 1983). Alternatively, some researchers take the method of sampling the plant tissues long after grafting in order to 'dilute' the effect of cutting wound (Liu et al., 2014), while others prefer to set

* Corresponding author at: 198 Shiqiao Rd, Hangzhou 310021, China. Fax: +86 571 86404223.
E-mail address: peixu@mail.zaas.ac.cn (P. Xu).

http://dx.doi.org/10.1016/j.scienta.2015.12.056
0304-4238/

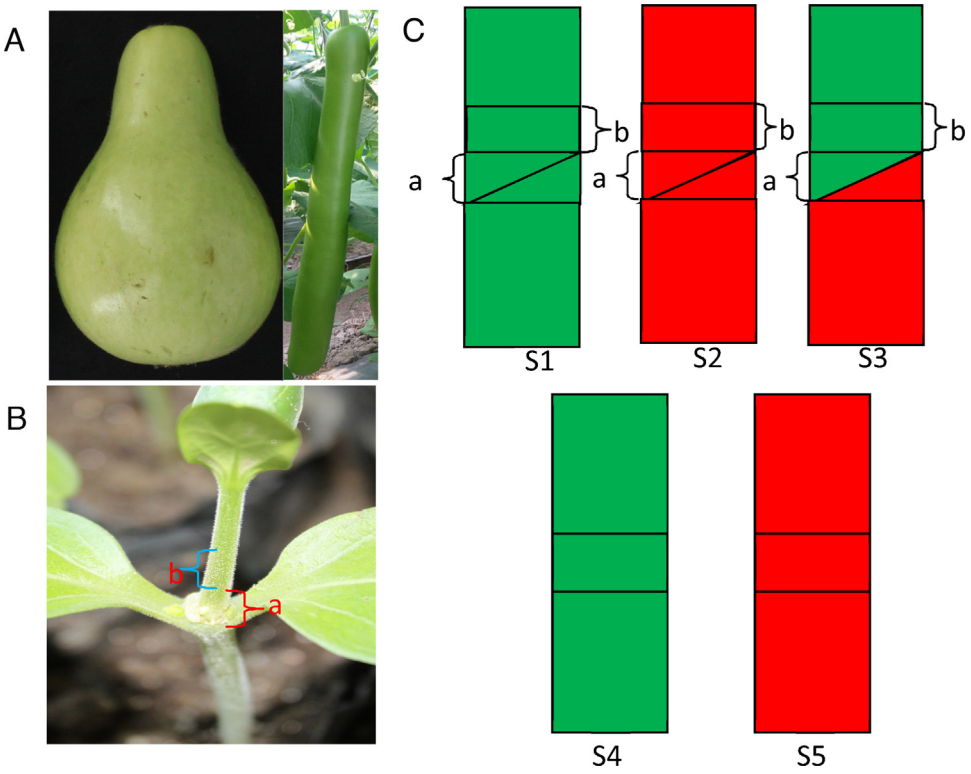

Fig. 1. Plant materials and experimental design for this study. (A) Fruit phenotypes of YZ (left) and HC (right). (B) Graft union (a) and the upper vicinity (b) that were sampled for proteomic analyses. (C) Illustration of the different scion-rootstock combinations. S1: HC/HC homo-grafts; S2: YZ/YZ homo-grafts; S3: HC/YZ hetero-grafts; S4: ungrafted HC; S5: ungrafted YZ.

up a homo-grafted control (Cookson and Ollat, 2013). However, to our knowledge, there are no currently published data using proteomic approaches justifying the effective of the later methods in discriminating the effects of hetero-graft *per se* from cutting wound in vegetable crops.

Proteins are effector molecules in cells that are more directly related to biological processes than mRNAs (Zieske., 2006). Whereas great progress has been achieved in graft biology, relatively few studies are from the proteomic perspective. Isobaric tags for relative and absolute quantitation (iTRAQ), a recently developed proteomic technique, over-performs traditional 2D-gel technique in many aspects including sensitivity, precision and sample throughput (Komatsu et al., 2014; Wu et al., 2006; Wiese et al., 2007). In this study, iTRAQ coupled with MS mass was employed to compare the proteomic profiles of hetero-grafted, homo-grafted and non-grafted bottle gourd plants. Our results demonstrated that effects of grafting wound and hetero-grafting *per se* were readily distinguishable at the proteome level. We also revealed apparent tissue and genotypic differences in the effects of grafting wound and hetero-grafting *per se*. The possible role of hydrogen peroxide as a signal molecule in hetero-grafting systems was highlighted.

2. Materials and methods

2.1. Plant materials

Plant materials used in this study included two bottle gourd varieties i.e. the “Hangzhou gourd” (HC) and “Yongzhen” (YZ). HC is a commercial cultivar with high yield, slender strait fruit shape favored by customers, but susceptibility to major field diseases including fusarium wilt and powdery mildew. YZ, on the contrary, is a landrace cultivar with pear-shaped fruits, high resistance to fusarium wilt and low temperature (Fig. 1A). Due to its excellent stress resistances, YZ is widely used as a rootstock for grafting with watermelon, cucumber and other bottle gourd genotypes in industry (Liu et al., 2013; Zhang et al., 2012). Both HC and YZ are inbred lines and their grafting survival rate is almost 100%.

2.2. Growth conditions and grafting manipulations

Seeds of each variety were sown in plastic pots (6 cm in diameter) filled with sterilized peat soil. After seed germination, the seedlings were kept in greenhouse under an ambient daily temperature of 30 °C and nightly temperature of 27 °C. Natural light/dark circles and normal water management were applied. The grafting was made at the two true-leaf stage using a slit-grafting method (Cushman., 2006). After inserting the trimmed scions into the slits of the rootstocks, the pots were enwrapped with transparent polyethylene bags and maintained for 7 days until sampling, a duration that is known to be enough for the graft union regaining cell adhesion between the two graft partners (Zhang et al., 2011; Hartmann et al., 2002). The two sampling sites for tissue collection were: the graft union (1 cm in length) and the immediate upper area (1 cm in length) along the stem of scion (Fig. 1B). The rootstock-scion combinations included HC/HC (homo-grafted, S1), YZ/YZ (homo-grafted, S2) and HC/YZ (hetero-grafted, HC was the scion cultivar and YZ was the rootstock cultivar, S3). Tissues were collected from 12 individuals of each combination. Ungrafted HC (S4) and YZ (S5) were also sampled (Fig. 1C). The experiments were repeated three times (on March, May and July of year 2014, respectively), and the tissues collected at each experiment were combined for iTRAQ assay.

2.3. Protein extraction, digestion and enrichment of lysine acetylated peptides

Samples were grinded in liquid nitrogen, transferred to 5-mL centrifuge tube and sonicated three times on ice using a high inten-