Identification of Polypeptides in Apple (*Malus x domestica* Borkh.) that may assist in determining plant stress related diseases

M. Krawitzky\(^1\), A. Blanco\(^1\), R. Oria\(^2\) and J. Val\(^1\)

**ABSTRACT**

The occurrence of nutrient-related disorders in apple fruit, such as bitter pit, cork and Jonathan spot, to name a few, are increasing problems of great importance for the fruit industry. Although bitter pit has been recognized and characterized for more than a century, there is little information related to the development of physiological disorders in fruit that trigger their occurrence. In a previous group study, a novel 18 kDa protein was found in bitter pit affected tissues, suggesting this protein band may be an indicator/marker for bitter pit disease in apples. No comparison was made between bitter pit diseased fruit and other fruit showing symptoms of another disease. The 18 kDa protein has been reported to contain Mal d 1, the major apple allergen that is believed to be pathogen and stress induced. Polypeptides from apple cultivar ‘Golden Delicious’ affected with bitter pit, and apple cultivar ‘Transparente Blanco’ affected with watercore were analysed with one-dimensional denaturing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine protein bands unique to each disease. Results indicate the 18 kDa protein was found not only in bitter pit affected apple tissues but also in watercore tissues. These findings do not dismiss the possibility that the 18 kDa protein band is an indicator for bitter pit, but potentially broadens the range the 18 kDa band may have as an indicator for disease or nutrient deficiency caused by plant stress.

**INTRODUCTION**

Physiological changes in apple (*Malus x domestica* Borkh.) whether caused by nutrient deficiencies, excess, or other abiotic origins are often identified by symptomatic affected areas appearing necrotic, sunken, or discoloured. Most com-

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1. Department of Plant Nutrition, Estación Experimental de Aula Dei (EEAD-CSIC), Avda. Montañana 1005. 50059 Zaragoza, Spain.
2. Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain.
commercially available apple cultivars are known to be susceptible to physiological related disorders such as bitter pit, scald, lenticel spot, and cork spot (Simons, 1968). These disorders cause significant loses for the fruit industry due to fruit alterations e.g. on organic acids stoichiometry, protein patterns, enzymatic activities and on cell death.

Bitter pit, one such apple disorder has been studied for more than a century. Bitter pit develops during post-harvest storage, caused partly to an imbalance of fruit calcium distribution, and is not yet completely understood. Although bitter pit prediction models (Lötze et al., 2008, Lötze et al., 2006, Retamales et al., 2000) have been created to understand where and why bitter pit occurs, these prediction models are often difficult to apply because of differences in fruit uniformity and maturity. Other methods which have utilized near infrared reflectance (NIR) spectroscopy (Nicolai et al., 2006) have examined bitter pit affected apples at harvest to attempt an early determination system, however NIR was unable to differentiate between bitter pit and corky tissue samples. Although bitter pit has been recognized and characterized for more than a century, there is little information related to the development of physiological disorders in fruit that trigger their occurrence.

In a previous group study (Val et al., 2006), a novel 18 kDa protein was found in tissues affected with bitter pit and ammonium oxalate injections (thought to induce bitter pit) and not in healthy tissue. Therefore the conclusion was given that this protein band was an indicator/marker for the bitter pit disorder in apples. However in this study, no comparison was made between bitter pit and another disorder, overlooking any possibility that the 18 kDa band may be something other than a protein which is present in bitter pit.

The 18 kDa protein in apples has been reported (Ebner et al., 1991, Vieths et al., 1994) to contain Mal d 1, the major apple allergen that is believed to be pathogen and stress induced.

Therefore the objective of this paper was to compare one-dimensional polypeptide profiles between bitter pit and samples collected from another apple disorder and to confirm if the 18 kDa apple allergen is present in both samples. If present, new conclusions can be made regarding the 18 kDa protein in apples (e.g. plant stress, nutrient deficiency).
MATERIALS AND METHODS

Plant material

Sampled apples were harvested late 2010 according to commercial agriculture practices and stored at 4°C (EEAD-CSIC) until May 2011. Apples were sampled according to their disorder and only affected tissue was sampled. Sample treatments (bitter pit, watercore, and healthy) were collected from apple cultivars ‘Golden Delicious’ and ‘Transparente Blanco.’ Collected tissue samples were lyophilized then stored at -20°C until analysis.

Protein Extraction

All samples were ground separately using an IKA A10 mill (Staufen, Germany) until a fine powder. All collected tissue from each apple and cultivar was pooled into one single sample and then homogenized. Approximately 8 mL extraction buffer (del Valle et al., 1998) was added to approximately 1 g of fine powder (1:9). Sample solutions were homogenized using an Omni-mixer (OCI Instruments, Kansas City, MO, USA) for approximately 30 s. Samples were then centrifuged for 30 min at 12,000g. The supernatant was then collected and filtered through a 0.45 μL filter. The filtrate was stored at -20°C until analysis.

Denaturing Polyacrylamide Gel Electrophoresis

Proteins were separated by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine protein bands unique to each disorder. Mini-gel matrices were prepared in two phases: 12-16% resolving and 4% stacking gels using 1.5mm gel plates. Gels were run in a mini-protean 3 and mini-protean tetra cell. (Bio-Rad).

Total amount of protein was quantified using total protein colorimetric assays (Bio-Rad, SERVA.DE).

Gels were stained for approximately 120 min with Oriole fluorescent gel stain (Bio-Rad). The Molecular weights for each band separated were determined using Gel-Pro Analyzer software (version 4 Media Cybernetics, L.P). A low-range standard (Bio-Rad) was used to calculate molecular weight. More than fifty gels, each with ten wells (wells 1/10 buffer) were used to determine molecular weight and present protein bands.
RESULTS AND DISCUSSION

Results (Fig. 1) indicate the 18 kDa protein was found not only in bitter pit (lanes 2,6) apple tissue but also in watercore tissue (lanes 4,5,8). Healthy apples samples lanes 1 and 7 did not have an 18 kDa protein band. Bitter pit and watercore samples shared similar protein bands at 20, 25.9, 32.9, 38.4, 41.9, 43.9, 56.2, 63.5, 69.2, 78.1 kDa.

The 18 kDa protein in this study was found both in bitter pit and watercore sampled apple tissues. In previous studies (Val et al., 2006) bitter pit affected apples were compared against healthy apples. Results from this study confirm that the 18 kDa protein was present in bitter pit but not in healthy apples, a result which our studies confirmed. However, in this study the presence of the 18 kDa protein denoted bitter pit, a result that cannot be as the 18 kDa protein was also found in watercore apple samples (our study). These findings do not dismiss the possibility that the 18 kDa protein band as an indicator for bitter pit, but potentially broadens the range the 18 kDa band may have as an indicator for disease or abiotic disorders. Another consideration is the effect of cultivar. Several cultivar studies have suggested that Golden Delicious contains greater concentrations of the 18 kDa protein when compared to other cultivars (Hsieh et al., 1995). In our study ‘Golden Delicious’ samples contained greater concentrations of the 18 kDa protein when compared to ‘Transparente Blanco.’ Also, time in storage most likely played a part in the development of the 18 kDa protein. Several papers have reported an increase in the 18 kDa protein in storage over time (Hsieh et al., 1995, Vieths et al., 1994) suggesting ripening should be considered in the development of the 18 kDa protein.

CONCLUSION

Bitter pit has long been studied due to its economic impact; more recently the associated 18 kDa Mal d 1 protein due to its importance in human health. Previous articles have suggested this protein may have been an indicator for bitter pit, but this article has shown otherwise. The 18 kDa protein band, most likely is not only an indicator for bitter pit but also for other diseases or plant stresses.

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BIBLIOGRAPHY


Better Pit - Lane 2, 6
Watercore - Lanes 4, 5, 8
Healthy - 1, 7

Fig. 1.
Sr. José Carlos Martínez Giménez  
Responsable Unidad  
Estación Experimental de Aula Dei  
EEAD-CSIC  

Lleida, 18 de febrero de 2014

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