

# ITS Region- An Efficient Genetic Tool to Identify Non-hybrid Butternut for Butternut (*Juglans cinerea* L.) Conservation

Bo Li, Keith E. Woeste

Forestry and Natural Resource, Purdue University

## Abstract

Butternut (*Juglans cinerea* L.), also called white walnut, is an ecologically and economically valuable tree. It is currently threatened by butternut canker disease that infects and kills butternut trees throughout the natural range of the species. Butternut has been listed as a "species of Federal concern" by the U.S. Fish and Wildlife Service. Hybridization of butternut and Japanese walnut has made butternut conservation a particularly difficult problem because hybrids of butternut and Japanese walnut [known as Buartnut (*J. cinerea* x *J. ailantifolia*)], tend to be more resistant to butternut canker, are very common, and are virtually indistinguishable from butternut.



BPO1sample from Indianapolis	30	IN
BPO2sample from Ostry-all places	15	MN
BPO3sample from Ostry-buttternut seedlings	47	MN
BPO4sample from Ostry-other butternut	55	MN
BPO5sample from Nolin River Nut Tree Nursery	4	KY
BPO6sample collected in summer, 2003	70	MA, IL,IN, OH,MN, NJ, MI
BPO7sample from HTIRC breeding program	30	IN, WI, MN, CA, MI, IL, WA,KY
BPO8sample from Whitewater-WI	305	WI
BPO9sample from Cascade-IA	166	IA

The ITS (internal transcribe spacer) region of the nuclear DNA includes three components: internal transcribed spacer 1, the 5.8S ribosomal RNA gene, and internal transcribed spacer 2. The ITS region has proven to be a useful tool for phylogenetic studies in many angiosperm families.(Fig.1) (Baldwin, et al., 1995).

PCR amplification of the ITS region has become a popular choice for phylogenetic analysis of closely related species and populations (e.g. Bayer et al. 1995). This popularity stems from the derivation of universal primers located in the coding regions flanking the ITS.

ITS region of samples of butternut, Japanese walnut and hybrid were amplified and digested with restriction enzymes to give diagnostic digestion products.(Fig.2)

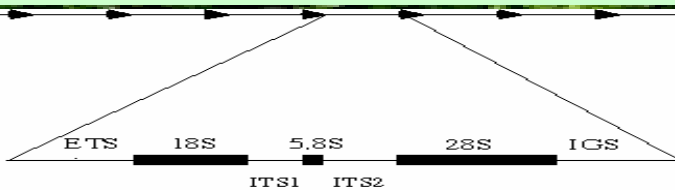


Fig 1. Organization of one rDNA array. Each unit consists of the rRNA genes 18S, 5.8S, and 28S. Spacers separate these genes, namely the external transcribed spacer (ETS), the internal transcribed spacers (ITS 1 and ITS 2) and the intergenic spacer (IGS).

Fig 2. ITS region amplifications and expected digestion products of butternut (J.c), buartnut (hybrid, J.a x J.c), Japanese walnut (J.a) and black walnut (J.n).

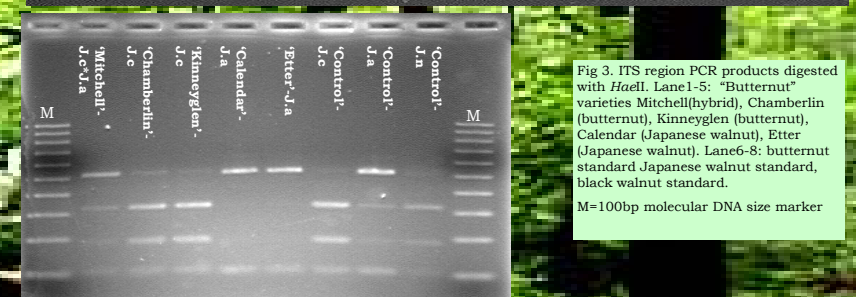
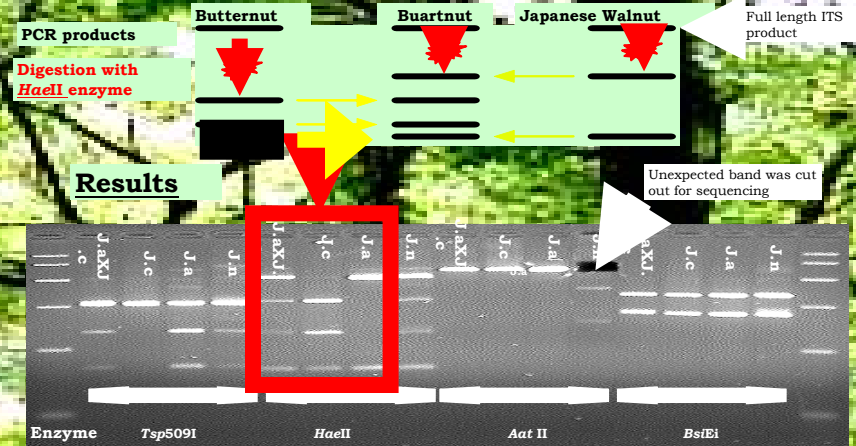


Fig 3. ITS region PCR products digested with *HaeII*. Lane1-5: "Butternut" varieties Mitchell(hybrid), Chamberlin (butternut), Kinneyglen (butternut), Calendar (Japanese walnut), Etter (Japanese walnut). Lane6-8: butternut standard Japanese walnut standard, black walnut standard.  
M=100bp molecular DNA size marker

## Discussion

Butternut conservation geneticists need to identify and remove hybrids from samples to obtain accurate estimates of genetic diversity and to evaluate resistance to butternut canker.

ITS DNA sequences of butternut, Japanese walnut, and black walnut were compared. Conserved regions flanking polymorphism were used to design primers for PCR amplification. We have identified sequence polymorphisms within the ITS region that can be used to distinguishing butternut from buartnut (Fig. 3). By using ITS polymorphisms we have determined that trees sold as butternut by one large, private nursery in the Midwest are in fact hybrids, e.g., the "butternut" varieties "Cobles 1", "Ayers", "Canoka" and "Bountiful".

ITS region is an efficient tool to identify non-hybrid butternut from hybrids between butternuts and Japanese walnut.

## Literature Cited

- Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S., Donoghue, M.J. 1995. The ITS Region of Nuclear Ribosomal DNA: A Valuable Source of Evidence on Angiosperm Phylogeny. *Ann. Missouri. Bot. Gard.* 82: 247-277
- Harden, R., Stanford, A.M. and Parks, C.R. *Am. J. Bot.* 87 (6), 872-882 (2000)
- Manos, P.S. and Stone, D.E. *Ann. Mo. Bot. Gard.* (2001)