J. Plant Biol. (2010) 53:395–399
DOI 10.1007/s12374-010-9128-5

ORIGINAL RESEARCH

Persistence of Genetically Modified Potatoes in the Field

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Received: 23 June 2010 / Revised: 8 September 2010 / Accepted: 9 September 2010 / Published online: 5 October 2010

Abstract Volunteers from genetically modified (GM) potatoes may pose an environmental problem if allowed to grow in the field after the annual crop is harvested. We tested whether they are more likely to produce volunteers than non-GM potatoes. Specifically, we compared the number of volunteers, number of tubers per plant, tuber size, and their vertical distribution in the soil. More volunteer plants came from non-GM potatoes than from GM potatoes, but the number and size of tubers were similar between the two. Vertical distribution of the tubers differed significantly, with most non-GM tubers being found in shallower soil (<2 cm deep). Our results suggest that spontaneous GM volunteers may emerge and produce tubers to a degree similar to that of the non-GM plants. No viable volunteers emerged from GM tubers in the next growing season, probably deterred by winter frost and a period of low soil temperatures (below −2°C) at our study site. However, in regions with warmer climates, such GM volunteers may survive Winter and produce more plants the following year.

Keywords Genetically modified (GM) crop · Persistence · Potato · *Solanum tuberosum* · Tuber · Volunteer

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Introduction

The potato (*Solanum tuberosum* L. subsp. *tuberosum*) originated in Peru and is now grown worldwide. It belongs to the section *Petota*, which covers more than 180 species distributed mainly in Central and South America (Kapteijns 1993; Celis et al. 2004; Spooner et al. 2005). Volunteer potatoes may arise from tubers or tuber pieces that remain in the soil after harvest (Askew and Struik 2007). These volunteers may be problematic in agronomic production when they are better competitors than following crops, such as maize (Boydston 2004; Boydston et al. 2008) and onion (Williams et al. 2005). Volunteer potatoes may also sustain soil-borne pests and pathogens (Thomas 1983; Ellis 1992). Although leftover tubers may be killed by exposure to winter frost, some may survive and become weeds in areas where soil temperatures do not drop below −2°C (Lumkes and Sijtsma 1972; Lutman 1977; Boydston et al. 2006). Volunteers produced from genetically modified (GM) potatoes may be even more problematic if they serve as a potential source for transgene spread (Love 1994; Conner 2006). This movement of transgenes via pollen or seeds has been studied extensively. However, it has not been well recognized that transgenes may persist in a crop field or escape to natural habitats through vegetative reproduction, e.g., stolons or rhizomes (Lu 2008). If true, then left unmanaged, GM volunteers may cross with crop, weedy, or wild relative species to spread their transgenes.

Here, we tested whether the overwintering tubers of GM potato are more likely to persist as volunteers than those from non-GM stock. After growing plants modified to contain the drought-tolerance gene *ABF3* from April to July 2009, we observed some volunteers emerging in September. Overexpression of *ABF3* is known to increase tolerance to drought in *Arabidopsis thaliana* (Kang et al. 2002) and

rice (Oh et al. 2005), and to cold and drought in lettuce (Vanjildorj et al. 2005). If volunteer GM potatoes survive winter frost, they may be a potential source of transgene spread in the field during the next growing season. Concerns may then arise regarding yield decreases and quality control for following crops. Therefore, to assess the possible environmental risks of modified potato, we compared the number of volunteers and their tubers between GM and non-GM plants. We also examined the vertical distribution of tubers within the soil and the volume of tubers from both types of potatoes.

Materials and Methods

Climatic Data

We obtained daily air temperature measurements and data for soil temperatures recorded at 10-cm and 30-cm soil depths from April 2009 to April 2010, as monitored at the Cheongju Weather Station (Korea Meteorological Administration 2010).

Field Experiment and July Harvest

A GM potato genotype (Line 207, T_4) was derived from non-GM cultivar Jopoong to contain *ABF3*. This gene is controlled by the cauliflower mosaic virus 35S promoter and the *nos* terminator. The neomycin phosphotransferase II (*nptII*) gene was introduced for kanamycin selection. We grew GM potatoes in a confined field at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongwon-gun, Republic of Korea (36°43′04″N, 127°26′07″E; elevation, 37 m). Three equal-sized blocks were established that were mulched with black plastic film to control weeds. Tubers from GM and non-GM 'Jopoong' (168 each) were planted on 16 April 2009, at depths of 10-20 cm. Following normal agricultural practices, we randomly hand-harvested tubers from 15 GM and 15 non-GM plants per block on 20 July 2009. The number of tubers was tallied from each plant and the diameter of the longest axis was measured for each tuber.

On 14 September 2009, approximately 2 months after the harvest, we observed the first emergence of new potato shoots in the field. From then on, we marked the location of each plant with plastic tags and sampled leaf tissues for DNA analysis. This was repeated until no additional shoots were found, at the end of November 2009.

Detection of GM Potato by PCR

Genomic DNA was extracted from 100 mg of fresh leaf tissues using a Genomic DNA Extraction Mini Kit (Plant) YGP100 (RBC Bioscience Corp., Taiwan) according to the manufacturer's protocol. PCR was performed to confirm GM potato volunteers. CaSIG-po-F1 (5′-TCT ACA TAC ATC AGG CAC CA-3′) and CaSIG-po-R1 (5′-AGG TCA AAA TGA ATT GGA TG-3′) were designed to detect *CaSIG4* (size, 220 bp) as an internal PCR-positive control (Fig. 1a). Forward primer tNOS-F (5′-GTC TTG CGA TGA TTA TCA TAT AAT TTC TG-3′) and reverse primer tNOS-R (5′-CGC TAT ATT TTG TTT TCT ATC GCG T-3′) were designed to amplify the 151-bp *nos* terminator (Fig. 1b; Matsuoka et al. 2002). All primers were synthesized by Bioneer (Daejeon, Korea). PCR was conducted with a final volume of 50 μL that contained 5 μL of gDNA, 1 μL of a 10-mM dNTP mixture, 0.4 μL of Taq DNA polymerase (Solgent, Korea), 5 μL of 10× Taq buffer, and 1 μL of 10 pmole for each primer. Conditions for amplification included an initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C (*CaSIG4*) or 55°C (*nos*) for 30 s, and extension at 72°C for 50 s; followed by a final extension at 72°C for 10 min.

Harvest of Volunteer Potatoes

On 23 November 2009, we carefully removed the soil around the remaining stems to harvest volunteer potatoes from plants that had been identified as GM or non-GM by PCR. The number of tubers was recorded per plant. For each tuber, we measured the depth at which it grew and the lengths of its three principal axes (x, y, and z) with calipers. Tuber volume (V) was estimated as $V=¾\pi abc$, where a, b, and c are half of the lengths of those three axes (Wurr 1977). We then buried the tubers in the soil at the depths where they had been found. At the beginning of the next growing season (from March to May 2010), we surveyed the emergence of volunteer potatoes.

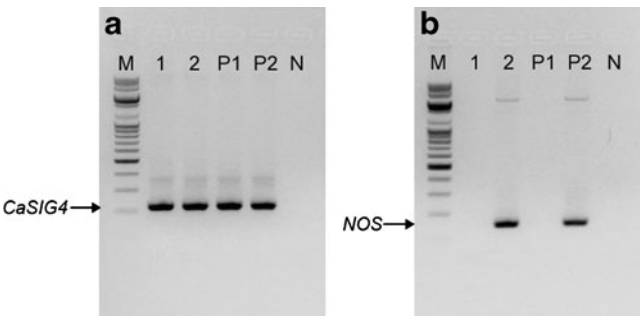

Fig. 1 Agarose gel electrophoresis of PCR products from potatoes, using primers for **a** *CaSIG4* and **b** *nos*. *M* 100-bp DNA ladder, *Lane 1* non-GM volunteer potato, *Lane 2* GM volunteer potato, *P1* positive control (non-GM potato), *P2* positive control (GM potato), *N* negative control (no DNA)

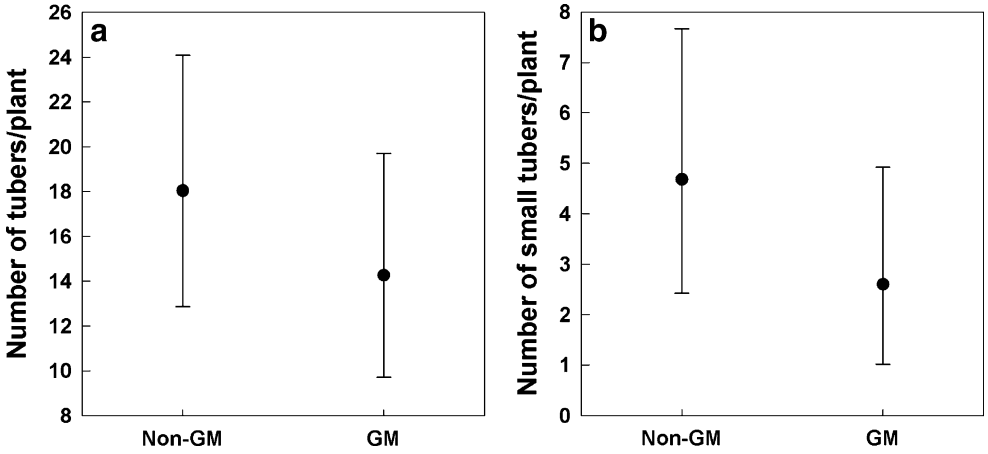

Fig. 2 Comparisons of **a** number of all tubers and **b** small tubers (<23 mm diam.) per plant between GM and non-GM plants grown until July. Data are means±95% confidence interval

Data Analyses

Using data obtained from the July harvest, we tested whether the number of tubers per plant differs between GM and non-GM stock. Data were square-root transformed and examined by analysis of variance with restricted maximum likelihood. Because our experiment utilized a randomized complete block design, the genotype (GM vs. non-GM) effect was tested against experimental error (block×genotype interaction).

Nonparametric tests were used for the data from our volunteer harvest because those values significantly deviated from a Gaussian distribution. We examined whether the number of tubers per plant and their tuber volume differ between GM and non-GM volunteers with a Wilcoxon test. The frequency distribution of tuber depths in the soil was also compared between GM and non-GM volunteers with a Kolmogov–Smirnov two-sample test. Statistical analyses were performed using JMP 8 (SAS Institute, Cary, NC) and STATISTICA (StatSoft, Tulsa, OK).

Results and Discussion

The July harvest revealed that non-GM potatoes had more tubers than did the GM plants (means of 18.0 and 14.3 tubers per plant, respectively), although this difference was not significant (P=0.163; Fig. 2a). Non-GM plants also had more small-sized (<23 mm diam.) tubers than did the GM plants (means of 4.7 and 2.6 tubers per plant, respectively), although this difference also was not significant (P=0.110; Fig. 2b). After the July harvest, 44 volunteers emerged from September to October 2009, with 16 being GM and 28 being non-GM volunteers, based on PCR analysis. Because farmers do not harvest small-sized tubers in practical agriculture, we would expect them to be the ones to develop more volunteer plants and, consequently, more tubers (Lutman 1977). Therefore, the greater number of small non-GM tubers left in the field may have produced more non-GM volunteers because the larger GM tubers had already been harvested in July.

From the November volunteer harvest, we found that non-GM volunteers produced slightly more tubers than the GM

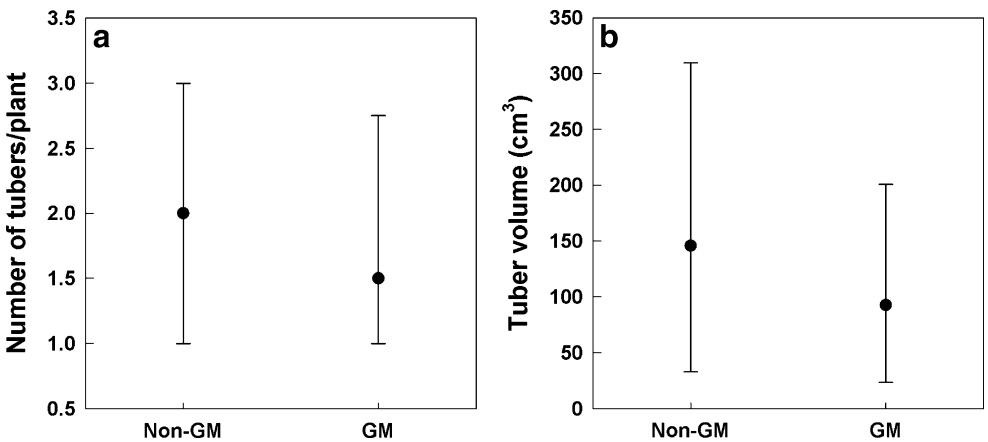

Fig. 3 Comparisons of **a** number of tubers per plant and **b** tuber volumes between GM and non-GM volunteers. Data are median and quartile range

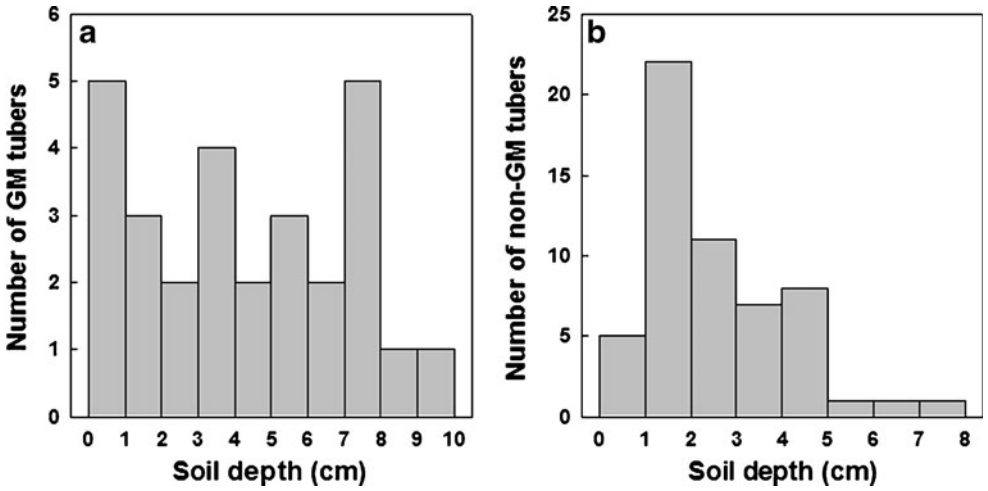

Fig. 4 Frequency distribution of tubers from (**a**) GM and (**b**) non-GM volunteers at various soil depths

(median=2.1 and 1.5 tubers per plant; Fig. 3a), although this was not significant (P=0.765). Interestingly, those non-GM volunteers also had a wider range of tuber numbers, up to eight per plant. Non-GM tubers also were larger than those from the GM (median=145.8 and 92.5 cm^3, respectively; Fig. 3b), although this difference was not significant (P=0.099). The number of non-GM tubers in our study generally agreed with values reported previously, including those from Lutman (1977) who recorded 1.3 to 1.9 tubers per plant from a wheat field in England. Pérombelon (1975) also counted 1.7 to 2.1 tubers per plant in a barley field in Scotland. In our experiments, the tuber count from GM volunteers was comparable to that from non-GM plants, suggesting that the former may emerge as GM plants and pose a problem in crop management.

The pattern of vertical distribution for tubers in the soil varied between GM and non-GM. Although tubers from both types were found at less than a 10-cm depth (Fig. 4), their frequencies of distribution differed significantly (P<0.05). GM tubers were rather evenly distributed from 0 to 8 cm (Fig. 4a), whereas a large proportion (~37%) of the non-GM tubers was found at 1 to 2 cm deep (Fig. 4b). On average, GM tubers were located 1.6 cm deeper into the soil. Although we cannot yet explain this variability, our results may suggest that patterns of vertical root growth differ between GM and non-GM potatoes.

GM potato volunteers may not be problematic if killed from winter frost. At our study site, low temperatures from 5 November 2009 to late that month damaged most of the shoots, and no volunteers emerged in Spring 2010. Potato tubers can be killed when experimentally treated at −2°C for 50 h (Lumkes and Sijtsma 1972) or when the minimum soil temperature reaches −2°C at the tuber depth (Boydston et al. 2006). The air temperature at our site dropped below 0°C on 6 December 2009 and was −10.2°C on 13 January 2010 (Fig. 5a). Furthermore, the soil temperature at 10 cm was below −2°C from 13 to 17 January 2010 (Fig. 5b), which may have killed all tubers at this depth. However, volunteer GM potatoes may thrive in the next growing season if the soil temperature remains above −2°C.

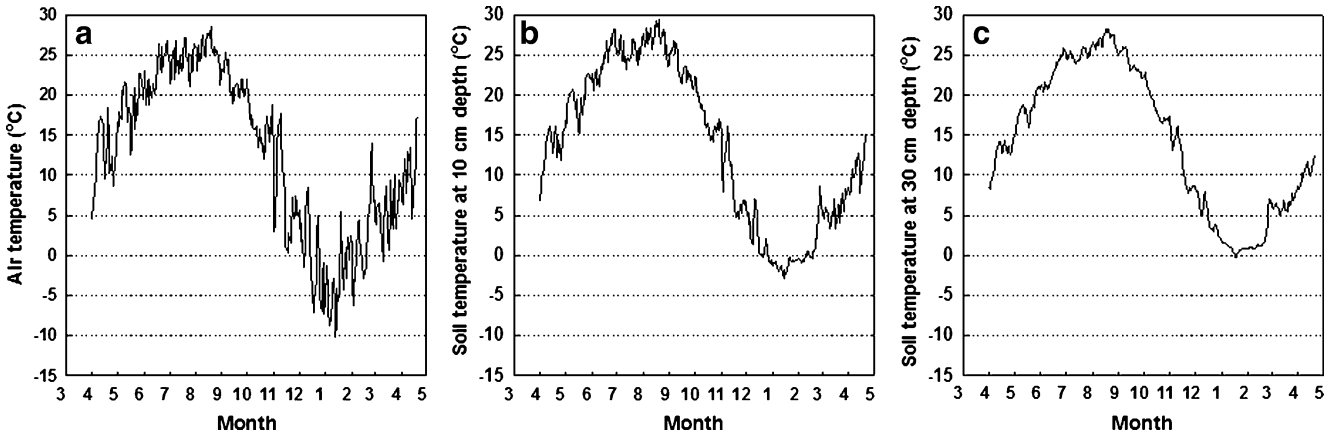

Fig. 5 (**a**) Air temperature and soil temperatures at (**b**) 10 cm and (**c**) 30 cm from April 2009 to April 2010 as recorded at Cheongju Weather Station, 8 km away from the study site

Our study site was left unmanaged after the July harvest and volunteers produced new tubers only at a depth of <10 cm. However, if the field had been plowed before wintertime, the remaining tubers may have become more deeply buried, where they could have then escaped freezing (Lumkes and Beukema 1973; Pérombelon 1975). Our experiment considered a soil temperature at 30 cm that did not drop below −2°C (Fig. 5c). Therefore, under such conditions, a potato field should not be plowed in Autumn (Lumkes and Beukema 1973). Tubers may also survive Winter in regions with warmer climates, e.g., Jeju Island in Korea. During our study period, the air temperature at Seoguipo on Jeju Island was below 0°C only once, and the minimum daily soil temperature at 10 cm was 4.7°C (Korea Meteorological Administration 2010). Crawley et al. (2001) have reported that non-GM and GM herbicide-tolerant potatoes survived for more than two consecutive years at four out of their eight study sites that had been established within the natural habitats of Cornwall and Berkshire, England. There, winter conditions are much milder than at our study site. In fact, at the Berkshire fields, GM and non-GM potatoes have survived for up to 10 consecutive years. Jeju Island is the second largest province for potato production in Korea (Korean Statistical Information Service 2010). Therefore, if the cultivation of GM potatoes is permitted in the near future, studies must focus on whether such plants can survive Winter and produce volunteers that harbor the transgene(s) under warmer climatic conditions.

Acknowledgements This research was supported by grants from the KRIBB Research Initiative Program and the Crop Functional Genomics Center (CG3212).

References

Askew MF, Struik PC (2007) The canon of potato science: 20. Volunteer potatoes. Potato Res 50:283–287

Boydston RA (2004) Managing volunteer potato (*Solanum tuberosum*) in field corn (*Zea mays*) with carfentrazone-ethyl and dicamba. Weed Technol 18:83–87

Boydston RA, Seymour MD, Brown CR, Alva AK (2006) Freezing behavior of potato (*Solanum tuberosum*) tubers in soil. Amer J Potato Res 83:305–315

Boydston RA, Collins HP, Alva AK (2008) Control of volunteer potato (*Solanum tuberosum*) in sweet corn with mesotrione is unaffected by atrazine and tillage. Weed Technol 22:654–659

Celis C, Scurrah M, Cowgill S, Chumbiauca S, Green J, Franco J, Main G, Kiezebrink D, Visser RGF, Atkinson HJ (2004) Environmental biosafety and transgenic potato in a centre of diversity for this crop. Nature 432:222–225

Conner AJ (2006) Biosafety evaluation of transgenic potatoes: gene flow from transgenic potatoes. In: Huang S-C, Chen S, Lin C-Y (eds) Ecological and Environmental Biosafety of Transgenic Plants. Proceedings of International Symposium. Taiwan Agricultural Research Institute, Taichung, Taiwan, pp 127–140

Crawley MJ, Brown SL, Hails RS, Kohn DD, Rees M (2001) Transgenic crops in natural habitats. Nature 409:682–683

Ellis PJ (1992) Weed hosts of beet western yellows virus and potato leafroll virus in British Columbia. Plant Dis 76:1137–1139

Kang J-Y, Choi H-I, Im M-Y, Kim SY (2002) *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell 14:343–357

Kapteijns AJAM (1993) Risk assessment of genetically modified crops. Potential of four arable crops to hybridize with the wild flora. Euphytica 66:145–149

Korea Meteorological Administration (2010) Climate data. http://www.kma.go.kr/gw.jsp?to=weather_main.jsp. Accessed 28 April 2010

Korean Statistical Information Service (2010) Statistical Database. http://kosis.kr/nsp/index/index.jsp. Accessed 27 April 2010

Love SL (1994) Ecological risk of growing transgenic potatoes in the United States and Canada. Amer Potato J 71:647–658

Lu B-R (2008) Transgene escape from GM crops and potential biosafety consequences: an environmental perspective. Collection Biosafety Rev 4:66–141

Lumkes LM, Beukema HP (1973) The effect of cultivation procedure on the liability to freezing of ground-keepers. Potato Res 16:57–60

Lumkes LM, Sijtsma R (1972) Mogelijkheden aardappelen als Onkruid in volggewassen te voorkomen en/of te bestrijden. Landb Planten 1:17–36

Lutman PJW (1977) Investigations into some aspects of the biology of potatoes as weeds. Weed Res 17:123–132

Matsuoka T, Kuribara H, Takubo K, Akiyama H, Miura H, Goda Y, Kusakabe Y, Isshiki K, Toyoda M, Hino A (2002) Detection of recombinant DNA segments introduced to genetically modified maize (*Zea mays*). J Agric Food Chem 50:2100–2109

Oh S-J, Song SI, Kim YS, Jang H-J, Kim SY, Kim M, Kim Y-K, Nahm BH, Kim JK (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351

Pérombelon MCM (1975) Observations on the survival of potato groundkeepers in Scotland. Potato Res 18:205–215

Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. Proc Natl Acad Sci USA 102:14694–14699

Thomas PE (1983) Sources and dissemination of potato viruses in the Columbia Basin of the northwestern United States. Plant Dis 67:744–747

Vanjildorj E, Bae T-W, Riu K-Z, Kim SY, Lee H-Y (2005) Overexpression of *Arabidopsis* ABF3 gene enhances tolerance to drought and cold in transgenic lettuce (*Lactuca sativa*). Plant Cell Tiss Org 83:41–50

Williams MM II, Ransom CV, Thompson WM (2005) Duration of volunteer potato (*Solanum tuberosum*) interference in bulb onion. Weed Sci 53:62–68

Wurr DCE (1977) Some observations of patterns of tuber formation and growth in the potato. Potato Res 20:63–75