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A STUDY OF COMPARATIVE ANALYSIS AMONG DNA CONTENT IN DRUPE, POME, HESPIRIDIUM, PEPO AND COMPLEX FRUITS

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ABSTRACT
Genomic DNA extraction is an important aspect of plant molecular biological research. The objective of the study was to recommend the cheap and efficient genomic DNA extraction method for some economically important fruit species of Sri Lanka. The modified plant genomic DNA extraction methods explained bydrupe, pome, hespiridium, pepo and complex fruits extraction kit (Qiagen) method were applied with nine different fruit species such as *Carica papaya* (Papaya), *Musa aouminata* (Banana), *Scolytus unipinosus* (Grape), *Psidium guajava* (Guava), *Malus angustifolia* (Apple), *Citrus aurantiam* (Orange), *Manilk arazapota* (Whitelead), *Pyrus commonis* (Pear), *Anaras conassus* (Pineapple).Based on the quantity of the extracted genomic DNA tested by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer, quality determined by the ratio of A260 / A280 and the amplifiable quality of DNA determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V, the method explained by Cheng et al and the Genomic DNA extraction kit yielded good quality DNA with satisfactory concentration for all the fruit species tested. Therefore the modified method of Cheng et al, 1987 could be recommended for the efficient and cost effective DNA extraction from fruit species instead of the commercially available expensive and chemically hazardous DNA easy plant kit method.

KEYWORD: *Carica papaya, Musa aouminata, Scolytus unipinosus, Psidium guajava and etc.*

INTRODUCTION
Maturity at harvest is the most important factor that determines storage-life and final fruit quality. Immature fruits are more subject to shriveling and mechanical damage, and are of inferior flavour quality when ripe.[1] Overripe fruits are likely to become soft and mealy with insipid flavour soon after harvest. Fruits picked either too early or too late in their season are more susceptible to postharvest physiological disorders than fruits picked at the proper maturity. All fruits, with a few exceptions (such as pears, avocados, and bananas), reach their best eating quality when allowed to ripen on the plant. However, some fruits are usually picked mature but unripe so that they can withstand the postharvest handling system when shipped long-distance. Most currently used maturity indices are based on a compromise between those indices that would ensure the best eating quality to the consumer and those that provide the needed flexibility in marketing.[2] The demand for tropical fruits has increased more than 40% during the last decade (Food and Agriculture Organization of the United Nations (FAO), 2010) as consumers seek healthy and more diverse food products. DNA is an almost universal genetic material, and that genes present in simple viruses, bacteria, plants, and animals are all made of DNA. It was a very long polymer made up of millions of nucleotides. The living cell is an extraordinarily complicated entity producing thousands of different macromolecules and harboring a genome. The methods of molecular biology depend upon an understanding of the properties of biological macromolecules. The systematic comparison of different animal genomics gives a chance of identifying genetic basis for diversity. We are fast entering a golden era of comparative genome analysis. Methods used to isolate the DNA depend on the source, age and size of the sample.[3] Principle behind the separation of DNA which is present in the cells is to make the DNA free from the other cellular components. Isolation of DNA is needed for the genetic analysis, which is used for scientific,

medical or forensic purpose. Scientists use DNA in a number of applications, such as introduction of DNA into the cells and animals or plants, or for diagnostic purposes.[4] Many protocols have been used for isolation of plant DNA, but because of chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some case fail to respond to the same protocol. Plants contain an array of secondary metabolites. The objective of the current study was to establish a DNA extraction procedure. Ten separate plant species were collected---their fruits were chopped in mortar and pestle and transferred for DNA extraction. After extraction of DNA, DNA presence was tested by Agarose gel electrophoresis. With Nano Drop Machine, the DNA content was measured.

DNA quantification using Nano Drop

[5]Nano Drop Spectrophotometer was used in current experiment. Nano Drop Spectrophotometer measures 1 micro ml samples with high accuracy and reproducibility. The full spectrum (220 nm-750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the Nano Drop Spectrophotometer has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

A 1 micro ml sample is pipetted onto the end of a fiber optic cable (the receiving fiber). A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. The gap is controlled to both 1 mm and 0.2 mm paths. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light after passing through the sample. The instrument is controlled by PC based software, and the data is logged in an archive file on the PC. The Nano Drop Spectrophotometer measures 1 ul samples with high accuracy and reproducibility. The full spectrum (220nm-750nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the Nano Drop Spectrophotometer has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Nano Drop Machine: The Sample Retention System

With the sampling arm open, the sample pipetted onto the lower measurement pedestal.[6] The sampling arm closed and initiated a spectral measurement using the operating software on the PC. The sample column is automatically drawn between the upper and lower measurement pedestals and the spectral measurement made. When the measurement is complete, the sampling arm opened and the sample wiped from both the upper and lower pedestals using a soft laboratory wipe. Simple wiping prevents sample carryover in successive measurements for samples varying by more than 1000 fold in concentration.

MATERIALS AND METHODS

Collection and preservation of fruits

The nine type fruit was collected in the kawran bazaar by the professional shop keeper. They made fruits in formalin and transported for marketing all over the country. We purchased about 5kg (mixed fruit) from Kawran Bazaar, Tejgaon, Dhaka in the very early morning from some fresh fruits.

Harvesting of fruits

The perched fruits was put the different fruit in different way. Cutted one type fruit into small pieces and use the blender for blending these fruits under liquate nitrogen. After blending collected the juice around 20ml and continues these process another 8types of fruit. These nine type fruit juice was preserved in refrigerator (-4^0C) until use.

Chemicals

GPX1 Buffer (optimized for samples with high polysaccharide content),GP1, GP2, GP3 Buffers, RNase A,W1 Buffer, Wash Buffer, Elution Buffer (10 mMTris-HCl, pH8.5 at 25ºC), agarose, buffer TAE (40 mMTris-acetate, 1 mM EDTA) and TBE (45 mMTris-borate, 1 mM EDTA), ethidium bromide (EtBr), EDTA. Water was purified using a distillation plant in our research laboratory. All buffer solution was filtered through a 0.4µm pore size membrane filter from Advantec^R (Japan). All other chemicals and reagents were obtained from commercial sources and were of the highest analytical grade available.

Preparation of Elution Buffer

125ml of 1M Imidazole solution was taken into 500mL graduated cylinder and Carefully pour the measured Imidazole into the 1L bottle.5mL of 1M Tris was taken into 5mL graduated cylinder and Carefully pour the measured Tris into the 1L bottle.1mL of EDTA was taken into 5mL graduated cylinder and Carefully pour the measured EDTA into the 1L bottle.369mL of MilliQ water was taken into500mL graduated cylinder and Carefully pour the measured MilliQ water into the 1L bottle. Then Cap these bottles securely and swirl the bottle to mix. Label the bottle Elution buffer, with the initials and the date. Auto clave the solution on a liquid cycle. (Refer to "Autoclaving stock solutions.").

DNA Quantification using Nano Drop

The Thermo Scientific Nano Drop™ 1000 Spectrophotometer measures 1 micro mlsamples with high accuracy and reproducibility.[7] The full spectrum

(220 nm-750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the Nano Drop 1000 Spectrophotometer has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Reagent

Tris-HCl, EDTA, Triton X-100 and 5µl RNase A (10mg/ml) was added into the sample tube and mixed by vortexing. Tris-HCl, EDTA, Triton X-100 are used for the purpose of breaking open cells. Tris-HCl, EDTA, Triton X-100 are added to break up membrane structures.

Tissue Dissociation

50 mg of plant tissue was grinded under liquid nitrogen to a fine powder. It was transferred into a microcentrifuge tube.

Lysis

[8]The mixture was incubated at 65° C for 10 minutes to weaken the cell walls and to lyse.100 µl lysis buffer was added and mixed by vortexing. The lysis buffer contains sodium hydroxide (NaOH) and the detergent Sodium Dodecyl (lauryl) Sulfate (SDS). SDS is to solubolize the cell membrane. NaOH helps to break down the cell wall, but more importantly it disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the genomic DNA SDS also denatures most of the proteins in the cells, which helps with the separation of the proteins. The closed tube was placed in the ice bath using forceps to hold the tube. The tube was kept in the bath for three minutes to freeze. All cells, the basic structural and functional unit of life, consist of living material bounded by layers of membranes made of lipids, proteins, and some other compounds. Cell lysis is the first step in the process of DNA purification. The DNA genome contains all the genetic information of an organism, and is protected from the external environment by the cell membrane. In order to release the genetic material for study and analysis, cells must be broken open, or lysed. There are several methods available for cell disruption including physical and chemical techniques. For this DNA extraction, freeze-thaw was used because it is a very common method used to lyse plant tissue cells. Cell lysis was followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, a filter column was placed in a 2 ml Collection Tube. The mixture from previous step was applied into the Filter Column. The filter column was centrifuged at full speed (13,000 rpm) for 3 minutes. The filter column was discarded and clarified supernatant was carefully transferred in Collection Tube to a new microcentrifuge tube.

DNA Binding

A GD Column was used as 2 ml Collection Tube.700µl the mixture (including any precipitate) was applied from previous step into the GD Column. The solution was centrifuged at full speed (13,00 rpm) for 2 minutes. The flow through was discarded in Collection Tube. Spin column-based nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions.

Washing

400µl of wash buffer was added into the Gd Column. Again it was centrifuged at 10,000 xg (13,00 rpm) for 30 seconds. The flow through was discarded and placed back in the Collection Tube.600µl of Wash Buffer was added into the Gd Column. It was centrifuged at 10,000 xg (\13,00 rpm) for 30 seconds. The flow through was discarded and returned into the 2ml Collection tube. It was centrifuged again for 3 minutes at full speed to dry the Column matrix.

DNA Elution

Standard elution volumn is 100 µl. If less sample volumn is used. The elution volume was reduced (30-50 µl) to increase DNA concentration. If higher DNA yield is required, the elution step was repeated to increase DNA recovery and the total elution volume to about 200 µl.The dried GD Column was transferred into a clean 1.5 ml microcentrifuge tube. 100 µl of preheated Elution Buffer was added onto the center of the column matrix. For 3.5 minutes it was kept standing until Elution Buffer absorbed by the matrix. It was centrifuged at 13,000 rpm for 30 seconds to elute purified DNA. The composition of Elution Buffer is:10 mMTris-Cl, pH 8.5

Determination of Agarose gel electrophoresis of Extracted DNA

[9]Agarose gel electrophoresis was used for the separation of the DNA fragments by using 0.7% of agarose. The sample DNA which was isolated from various sources are mixed with ethidium bromide, a marker dye and then loaded well in agarose gel which was then kept in position in the electrophoresis chamber filled with buffer and current was applied (typically 100 V for 30 min). The marker dye had a low molecular weight and migrated faster than the DNA. When the marker dye approached the end of the gel, the current was stopped and viewed under ultra violet light (Moyo et al 2008).

Preparation of the Gel

Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using aw/v percentage solution. The concentration of agarose in a gel will depend on the sizesof the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mMTris-

acetate, 1 mM EDTA) and TBE (45 mMTris-borate, 1 mM EDTA).Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min, followed by destaining in running buffer for an equal length of time. Allow the agarose to cool either on the benchtop or by incubation in a 65°C water bath. Failure to do so will warp the gel tray. Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box alternatively, the gel can also be wrapped in plastic wrap and stored at 4°C until use.

Setting up of Gel Apparatus and Separation of DNA Fragments

Add loading dye to the DNA samples to be separated (Fig. 2). Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel. Program the power supply to desired voltage (1-5V/cm between electrodes).Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working. Remove the lid. Slowly and carefully load the DNA sample(s) into the gel. An appropriate DNA size marker should always be loaded along with experimental samples. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads).

Observing Separated DNA fragments

When electrophoresis has completed, turn off the power supply and remove the lid of the gel box. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system).

RESULTS AND DISCUSSION

Table 1: Quantity of Extracted fruits using Nano Drop Machine

Plant Speacies	DNA Yield
Carica papaya(Papaya)	5.3ng/µl
Musa aouminata(Banana)	11.8ng/µl
Scolytusunipinosus (Grape)	6.3ng/µl
PsidiumGuajava(Guava)	3.3ng/µl
Malus angustifolia(Apple)	3.5ng/µl
Citrus aurantiam(Orange)	5.7ng/µl
Manilkarazapota(White lead)	6.3ng/µl
Pyruscommonis(Pear)	1.4ng/µl
Anarasconassus(Pineapple)	2.8ng/µl

Among the fruit species tested, fresh fruits of *Carica papaya* (Papaya), yielded maximum amount of DNA with overall mean of 5.3ngµL-1, *Musa aouminata* (Banana), *Scolytusunipinosus* (Grape), *PsidiumGuajava* (Guava), *Malus angustifolia* (Apple) *Citrus aurantiam* (Orange) *Manilkarazapota* (White lead) *Pyruscommonis* (Pear) *Anarasconassus* (Pineapple) maximum amount of DNA with over all mean of 11.8NG/µL, 6.3ng/µl, 3.3ng/µl, 3.5ng/µl, 5.7ng/µl, 6.3ng/µl, 1.4ng/µl,2.8ng/µl for nine type of fruits, where as modified Cheng et al. (2003) method yielded maximum amount of DNA with overall mean of 768 ngµL-1 and the modified Doyle and Doyle (1987) method yielded an overall mean of 337 ngµL-1 . Amount of DNA yield was higher in the DNA extraction kit method and lower in the modified Doyle and Doyle method.

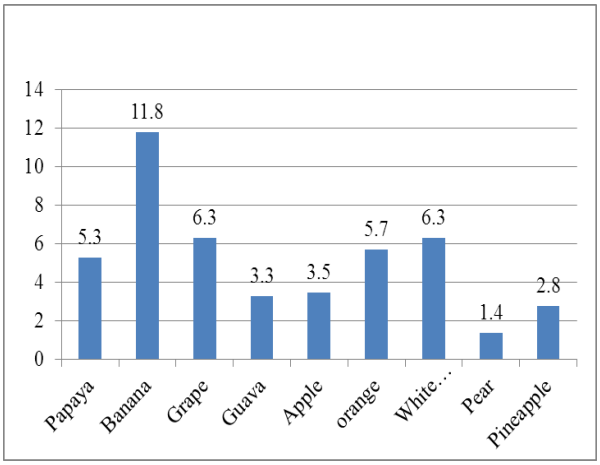

Figure: Quality means DNA the Different extracted fruit.

The DNA content in drupe fruits was significantly more that in pome, hespiridium, pepo and complex fruits (two tailed t-test value 0.26).

Agarose gel electrophoresis of extracted DNA

Gel running of samples from all the fruit species using all these methods showed considerable amount of amplifiable quality DNA except, *Musa aouminata* (Banana), *Scolytusunipinosus* (Grape), *PsidiumGuajava* (Guava), *Malus angustifolia* (Apple), *Citrus aurantiam* (Orange) *Manilkarazapota* (White lead), *Pyruscommonis*

(Pear), *Anarasconassus* (Pineapple), *Carica papaya* (Papaya)The present study showed that there was variation in time required for different DNA extraction kit method. On the contrary, modified Doyle and Doyle et al. method involved several time consuming extraction steps and took more than 10 hours to finish the entire processes. Among these methods investigated, all extracted amplifiable DNA from all the nine plant species with some exceptions of with modified *Musa aouminata* (Banana).

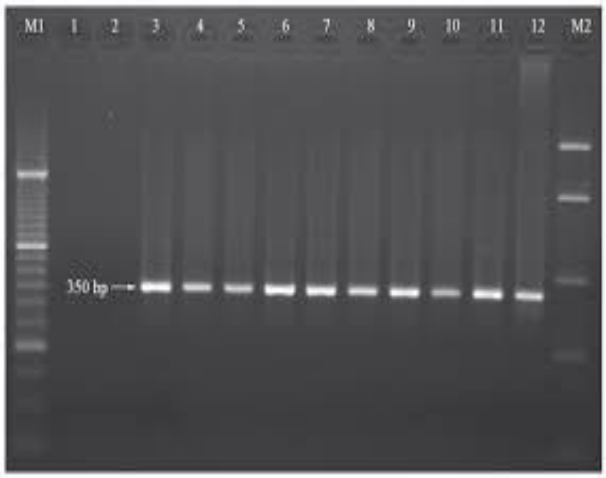

Figure: Gel showing the amplified PCR fragments of the genomic DNA extracted kit method from the fruits are *Musa aouminata* (Banana), *Scolytusunipinosus* (Grape), *PsidiumGuajava* (Guava), *Malus angustifolia* (Apple), *Citrus aurantiam* (Orange) *Manilkarazapota* (White lead), *Pyruscommonis* (Pear), *Anarasconassus* (Pineapple), *Carica papaya* (Papaya).

Agarose gel electrophoresis has proven to be an efficient and effective way of separating nucleic acids Agarose's high gel strength allows for the handling of low percentage gels for the separation of large DNA fragments. Molecular sieving is determined by the size of pores generated by the bundles of agarose in the gel matrix. In general, the higher the concentration of agarose, the smaller pore size.[10] Traditional agarose gels are most effective at the separation of DNA fragments between 100 bp and 25 kb. To separate DNA fragments larger than 25 kb, one will need to use pulse field gel electrophoresis, which involves the application of alternating current from two different directions. In this way larger sized DNA fragments are separated by the speed at which they reorient themselves with the changes in current direction. DNA fragments smaller than 100 bp are more effectively separated using polyacrylamide gel electrophoresis Unlike agarose gels, the polyacrylamide gel matrix is formed through a free radical driven chemical reaction. These thinner gels are of higher concentration, are run vertically and have better resolution. In modern DNA sequencing capillary electrophoresis is used, whereby capillary tubes are filled with a gel matrix. The use of capillary tubes allows for the application of high voltages, thereby enabling the separation of DNA fragments (and the determination of DNA sequence) quickly. Agarose can be modified to create low melting agarose through hydroxyethylation. Low melting agarose is generally used when the isolation of separated DNA fragments is desired. Hydroxyethylation reduces the packing density of the agarose bundles, effectively reducing their pore size. This means that a DNA fragment of the same size will take longer to move through a low melting agarose gel as opposed to a standard agarose gel. Because the bundles associate with one another through non-covalent interactions[9], it is possible to re-melt an agarose gel after it has set. EtBr is the most common reagent used to stain DNA in agarose gels. When exposed to uv light, electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to ground state. EtBr works by intercalating itself in the DNA molecule in a concentration dependent manner.[11] This allows for an estimation of the amount of DNA in any particular DNA band based on its intensity. Because of its positive charge, the use of EtBr reduces the DNA migration rate by 15%. EtBr is a suspect mutagen and carcinogen, therefore one must exercise care when handling agarose gels containing it. In addition, EtBr is considered a hazardous waste and must be disposed of appropriately. Alternative stains for DNA in agarose gels include SYBR Gold, SYBR green, Crystal Violet and Methyl Blue. Of these, Methyl Blue and Crystal Violet do not require exposure of the gel to uv light for visualization of DNA bands, thereby reducing the probability of mutation if recovery of the DNA fragment from the gel is desired. However, their sensitivities are lower than that of EtBr. SYBR gold and SYBR green are both highly sensitive, UV dependent dyes with lower toxicity than EtBr, but they are considerably more expensive. Moreover, all of the alternative dyes either cannot be or do not work well when added directly to the gel, therefore the gel will have to be post stained after electrophoresis. Because of cost, ease of use, and sensitivity, EtBr still remains the dye of choice for many researchers. However, in certain situations, such as when hazardous waste disposal is difficult or when young students are performing an experiment, a less toxic dye may be preferred. Loading dyes used in gel electrophoresis serve three major purposes. First they add density to the sample, allowing it to sink into the gel. Second, the dyes provide color and simplify the loading process. Finally, the dyes move at standard rates through the gel, allowing for the estimation of the distance that DNA fragments have migrated. The exact sizes of separated DNA fragments can be determined by plotting the log of the molecular weight for the different bands of a DNA standard against the distance traveled by each band. The DNA standard contains a mixture of DNA fragments of pre-determined sizes that can be compared against the unknown DNA samples. It is important to note that different forms of DNA move through the gel at different rates. Super coiled plasmid DNA, because of its compact

conformation, moves through the gel fastest, followed by a linear DNA fragment of the same size, with the open circular form traveling the slowest.

CONCLUSION

DNA technology has also had a major impact on the pharmaceutical industry, agriculture, disease therapy and even crime scene investigations. Let's take a closer look at the effects DNA technology has had on our world and the applications of such an important field of study. These are organisms that have genes from artificial means. GMOs are used for a variety of agricultural purposes, such as growing larger plants with higher yields, creating pest-resistant crops and improving the nutritional value of crops. For example, in India, a salt-resistant gene has been inserted into rice so that it can grow in water that is three times as salty as seawater! Another new variety of rice now exists that has very high beta-carotene levels to help reduce vitamin A deficiencies in certain parts of the world. One form of apple allergy, often found in northern Europe, is called birch-apple syndrome, and is found in people who are also allergic to birchpollen. Allergic reactions are triggered by a protein in apples that is similar to birch pollen, and people affected by this protein can also develop allergies to other fruits, nuts, and vegetables. Reactions, which entail oral allergy syndrome (OAS), generally involve itching and inflammation of the mouth and throat, but in rare cases can also include life-threatening anaphylaxis. This reaction only occurs when raw fruit is consumed—the allergen is neutralized in the cooking process. The variety of apple, maturity and storage conditions can change the amount of allergen present in individual fruits. Aricultural research has suffered from its own success. Politicians have concluded that the problem of agriculture has been solved and further research is unnecessary. Agricultural research now accounts for only 2% of the U.S. federal research and development budget, despite a 35% rate of return to society. Combined federal and state research expenditures have been flat at $2.5 billion for the past 20 years while private investment has grown rapidly, accounting for 60% of total expenditures by 1995. More than 20% of the research budget at state universities is from industry. The value of agriculture to society in the U.S. dwarfs its investment. Eighteen percent of American jobs are tied directly or indirectly to agriculture, as is 15% of the gross domestic product. Over 30% of U.S. agricultural products are exported, at a value of $56.5 billion; this is twice the value of our agricultural imports.

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