Nepal-Canada asparagus germplasm trade
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Product description

The product that is being proposed is an asparagus germplasm trade. The asparagus lines developed by the University of Guelph would be traded for germplasm samples from Nepal's universities and governmental organizations. One of the lines that may be sent to Nepal is the Guelph millennium cultivar of asparagus. This asparagus cultivar has a higher freezing tolerance compared to the jersey giant (JG) and UC157 (UC) cultivars (Kim, 2014). The asparagus cultivar Guelph millennium (GM) is also high in Rutin and Glutathione, and both are useful to improve health (Drinkwater, Tsao, Liu, & Wolyn, 2014). The GM asparagus can handle soil freezing down to -12c, which allows for the possibility of asparagus production to be pushed to colder regions of Nepal (Kim, 2014). One of the lines that Canada may receive is a sample of asparagus racemosus. This species has similar chromosome counts as the common asparagus (asparagus officinalis), and asparagus racemosus and asparagus officinalis have both tetraploid and diploid forms allowing for the possibility of future hybridization (Kanno & Yokoyama, 2011). The problem with this plan is that asparagus racemosus is in a sub genus that has four species incompatible with the common species, asparagus officinalis, so there may be some complications in using this species (Kubota, Konno, & Kanno, 2012). One of the diseases in asparagus officinalis is crown rot, which is caused by the species fusarium oxysporum (Elmer 1989). In an experiment with multiple plant species, researchers experimented on extracts of asparagus racemosus(Kaushik et al., 2014). The researchers have used these extracts on different species of fungi including Fusarium oxysporum and found that there was a moderate antifungal effect on the fusarium species with the asparagus racemosus extract(Kaushik et al., 2014).

Trade issues

One of the issues in this process is that there is the possibility of sending invasive pathogen, insect and plant species to Nepal through trade similar to the USA (Work, McCullough, Cavey, & Komsa, 2005). This means we will have to send the plant material to Nepal in a sterile medium. Soilless potting media will be ideal because the components of this medium are essentially sterile(Lang & Elliott, 1991). Another issue that may show up is the fact that plant material made from vegetative materials may have viruses (Fuller, Alston, & Golino, 2013). The virus removal methods include erratic virus distribution, chemotherapy, heat

treatment and meristem tip culture (Hollings, 1965). The erratic virus distribution method works because the amount of virus in different parts of the plant is not uniform so virus free pieces can be selected and cultured(Hollings, 1965). Chemotherapy does not really work because existing chemicals do remove enough viruses and damage the plant but heat treatment and meristem tip culture still work well (Hollings, 1965). There are also trade barriers in Nepal which may limit the flow of germplasm these include procedural barriers, institutional barriers, policy barriers and Structural barriers (Bishnu P. Sharma et al ., 2014). Procedural barriers are barriers are government developed systems made to control trade(Bishnu P. Sharma et al., 2014). Examples of Procedural barriers are certificates of origin and quality tests (Bishnu P. Sharma et al., 2014). Policy barriers would include prohibition and taxation of products such as the ban on forestry product exports (Bishnu P. Sharma et al., 2014; Nepal Ministry of Finance, 2016).. Institutional barriers are made when the delegation of responsibilities between governmental organizations is not well established, this is a rare phenomenon(Bishnu P. Sharma et al., 2014). Structural barriers are made by infrastructure and technology limitations (Bishnu P. Sharma, Shiva R. Adhikari, 2014). Examples in Nepal include the lack of electricity, limited free labour, and high transportation costs(Bishnu P. Sharma, Shiva R. Adhikari, 2014).

Introduction to Nepal

Nepal is a landlocked country surrounded by India and China and has regions of mountains, hills and terai(Berkat & Tazi, 2006; World Bank, 2014). The Nepali people have livestock as a source of income via the sale of milk, yoghurt, cheese, ghee, chhurpi, meat and eggs(World Bank, 2014). The common livestock in Nepal are yaks, cattle, goats, sheep and recently poultry(World Bank, 2014). This country has soil textures that consist mostly of sand in the crop soil types of Bari and Khet(Marx, 2012). The soil locations used for crops include corn, millet, vegetable, mustard and rice(Marx, 2012).The other crops produced in Nepal include wheat, barley pulses, sugar cane, ginger, cardamom, mango, papaya, banana, orange, lime, lemon, peach and plums. One of the common vegetables produced in Nepal is asparagus alongside sweet corn, potato, spinach, eggplant and tomatoes (Schnelle, 2012). The population of Nepal is 27.47 million with around 51.5% of the population being female(Agarwal, 2014). The population religious demographics is 86% Hindu, 8% Buddhist and 3% Muslim(Agarwal, 2014).

The inputs consumed by the Nepali people in terms of the nutrients nitrogen and phosphorous in tonnes has diminished from 2002-2008 before steeply going up in 2009-2014(FAO, 2016). Potash use has on the other hand remained stable during the 2002-2014 period(FAO, 2016). Undernourishment has steadily gone up from 1989-1999 it then took a small dip around the year 2000 before plateauing around 2001(FAO, 2016). From 2002-2016 the level of undernourishment has steadily gone down from around 4 million to 2 million(FAO, 2016). The prevalence of undernourishment has steadily gone down from 1989-2016 and is now below 10%(FAO, 2016). The average protein supply per day in Nepal has increased from around 55 g in 1989 to 65 g in 2011(FAO, 2016). The average amount of protein of animal origin plateaued around 9 g per day from 1989-2003(FAO, 2016). In 2004 the amount went up to around 10 g per day and plateaued during 2005-2011 and went up around 2012-2013 to 11 g per day (FAO, 2016). The present amount dietary energy supplied by cereals, roots and tubers has gone down from 77.5 to 72.5 in 1989-2011(FAO, 2016). The Average economic value of the food produced in Nepal has gone from around $150 to over $200 between 1989 and 2015(FAO, 2016).

Nepal economics

In Nepal there is an increased popularity in hybrid style seeds of vegetables such as cauliflower, cabbage tomatoes (Luitel, Subedi, & Khatri, 2008). This has resulted in a greater potential in generating more income from off-season hybrid vegetables (Luitel et al., 2008). This means that the economy of Nepal is ready for modern asparagus cultivars. The cultivars of the asparagus sent to Nepal will have to be produced in a way that reduces the prospect of pathogens and other invasive species (Work et al., 2005). The methods used to produce the plant samples would be made using heat treatment and/or meristem tip culture and soilless medium will be used (Hollings, 1965; Lang & Elliott, 1991). Heat treatment is one method used to produce virus free stock (Hollings, 1965). The way this works by using 34-54c hot air or hot water to actively growing tissue to remove viruses (Hollings, 1965). The other method is meristem tip culture, viruses cannot enter meristem tissue so plant samples made from this tissue is virus free (Hollings, 1965). The Gross Domestic Product (GDP) of Nepal in 2012 per capita was $690 USD(Agarwal, 2014).

Benefits to Canada

The benefit to Canada includes the possibility of trade with Nepal when it comes to their wild species germplasm. There are many species wild species of asparagus one of which is the species asparagus Racemosus (Jha, Shrestha, Upadhyay, Stimart, & Spooner, 1996). There are other species of asparagus like Asparagus filicinus and Asparagus gracilis (Shreshtha & Shreshtha, 1994). Researchers have done work on callas tissue in the species Asparagus officinalis and found that the samples can recover from gene bombardment and have the genes of interest be incorporated into the original genome and allow the transformation process to be completed (Kubota et al., 2012). There are still problems with this genetic modification system such as the fact that the resultant plants are unable to be crossed with other Asparagus cultivars, this problem could be corrected in future experiments (Kubota et al., 2012). In this case the germplasm of asparagus racemosus may be available for trade in exchange for the Asparagus officinalis germplasm. Another method that could be used for genetic engineering is the direct transfer of genes to protoplasts(Conner & Abernethy, 1996). Protoplasts are cells that have had their cell walls removed and have being made more exposed(Conner & Abernethy, 1996). The protoplast gene transfer process is started by dissolving the cell walls(Conner & Abernethy, 1996). The researchers then use a high voltage pulse, heat shock or the chemicals polyethylene glycol and calcium to allow some cell permeability (Conner & Abernethy, 1996). The cells are then mixed with the DNA of choice so that the protoplasts can incorporate the genes into their genome(Conner & Abernethy, 1996). The most common plant source used for the direct transfer of genes to protoplasts is callus cultures(Conner & Abernethy, 1996). The reason for the use of callus cultures in this method is that other sources of cells have high levels of genetic variation this method can make lines that are fertile(Conner & Abernethy, 1996).

Benefits to Nepal

The Guelph Millennium asparagus cultivar can handle soil freezing down to -20c allowing for asparagus production allowing an increase in land used for this crop (Kim, 2014). This also grants the possibility of crossing this variety with one of the Nepal local cultivars of Asparagus. The super male variants of Asparagus tend to have greater vigor then the conventional cultivars (Cakir & CAKIR, 2007). The use of these super male variants of Asparagus means that only male plants will appear in the field preventing seedlings from appearing and becoming a weed

problem (Ii, Uno, Kanechi, & Inagaki, 2012). The possible utilization of a cheap single step DNA extraction and multiplex PCR method would be sufficient to weed out false negatives and identify all male lines much easier in asparagus (Kaushik et al., 2014). This means that the Nepali government or universities even with a limited budget could potentially create cultivars using Guelph's germplasm and their own varieties to develop cultivars suitable to the Nepal climate. There seems to be an improvement in both longevity and disease resistance in all male lines of Asparagus officinalis in comparison to the female lines (Nikoloff & Falloon, 1990). The all-male lines of Asparagus officinalis also produce a higher yield in comparison to other cultivars of Asparagus officinalis (Kanno, Kubota, & Ishino, 2014). The asparagus cultivars contain antioxidents known as the caffeic and ferulic acids reduce the chance of cancer and reduce cholesterol levels(Lee et al., 2014).asparagus also contains other antioxidents such as rutin and glutathione(Drinkwater et al., 2014). Rutin can strengthen capillary walls; this reduces the hemorrhaging that comes with hypertension while glutathione reduces the risk of certain cancers(Drinkwater et al., 2014). For 5 spears of asparagus you can get 20 calories, 7% is potassium, 1% is carbohydrates, 8% is dietary fibre, 2 grams of protein, 2 grams of sugar, 10% Vitamin A, 15% Vitamin C, 2% Calcium and 2% Iron(FDA, 2016). One of the problems in Nepal is the soil erosion(Gardner & Gerrard, 2003). The soil erosion in the mid hill region of Nepal is 3.65 per year(Gardner & Gerrard, 2003). With soil erosion there has being a link between perennial crops reduced soil erosion(Vallebona, Mantino, & Bonari, 2016). The total erosion is highest under cool season cereals(Vallebona et al., 2016). The second highest soil erosion situation is under cool season forage(Vallebona et al., 2016). The third highest soil erosion situation is under mixed meadow(Vallebona et al., 2016). The system with the least amount of erosion under alfalfa (Medicago sativa) this shows that perennial crops can help reduce soil erosion(Vallebona et al., 2016). Crops that can act as hedge can also help reduce soil erosion and be edible(Poudel, Midmore, & West, 2000). The crops that can act as hedge crops include tea, lemon grass, pigeon pea, pineapple and asparagus(Poudel et al., 2000). Some of the soils of Nepal's hill region are sandy in nature so it is important to have a perennial crop that works in that condition(Gardner & Gerrard, 2003). The species asparagus officinalis can grow in sandy soil making a possible fit for Nepali hill regions(Roth & Gardner, 1989).

Material costs for Canada and Nepal

There are costs of using tissue culture to mass produce garden asparagus plants. The ingredients for the tissue culture mix of garden asparagus are sucrose, auxins such as IAA NAA and 2,4-D, gibberellin, ethylene, phloroglucinol and a MS medium with vitamins (Chen, 2015). The cost for the MS medium is $0.86 USD per L(Phytotechlab, 2016). The costs of D-sucrose is $3.09 USD per 30 g(Phytotechlab, 2016).The costs for IAA costs $14.60 USD per 5 g(Phytotechlab, 2016). The costs for NAA costs $14.60 USD per 100 ml (Phytotechlab, 2016). The costs for 2,4-D $14.60 per 100 ml(Phytotechlab, 2016).

Conclusions

The conclusion is that the asparagus germplasm trade that is being proposed is a win-win for both countries. Nepal's universities would get the asparagus lines developed by the University of Guelph such as the Guelph millennium cultivar the University of Guelph would get germplasm samples of Nepal's native asparagus species which may confer new and interesting traits of interest. The asparagus lines such as Guelph millennium have the high freezing tolerance compared to other cultivars(Kim, 2014; World Bank, 2014).

The asparagus cultivars like Guelph millennium (GM) are also high in antioxidents that are useful in improved human health(Drinkwater et al., 2014). The Guelph asparagus lines can handle soil freezing down to -12c allowing for the possibility asparagus production to pushed to colder regions of Nepal (Kim, 2014). Of the benefits to Canada the species asparagus racemosus is one such benefit. This species has chromosome populations as both tetraploid and diploid a character trait the common asparagus (asparagus officinalis) shares in common with asparagus racemosus (Kanno & Yokoyama, 2011). One of the problems with the plan to breed with this species is that asparagus racemosus in a sub genus that has four species incompatible with the crop species so there may some complications in using this species (Kubota et al., 2012). The prospect of using this species would however allow the university of Guelph to incorporate the moderate antifungal effect on the fusarium species found in asparagus racemosus biology(Kaushik et al., 2014).

Other benefits to Canada could include the possibility of trade with Nepal when it comes to their wild species germplasm. The species that could be traded include many species wild

species of asparagus such as asparagus Racemosus, Asparagus filicinus and Asparagus gracilis(Jha et al., 1996; Shreshtha & Shreshtha, 1994). Although interbreeding capabilities between these species and Asparagus officinalis is unknown genetic modification remains a possibility. The Research done on callas tissue in the species Asparagus officinalis has found that the samples of this species can recover from gene bombardment and have the genes of interest be incorporated into the original genome and allow the transformation process to be completed allowing for the possibility for superior cultivars of asparagus to be modified and sent out into the market (Kubota et al., 2012). One of the issues is that this current form of the genetic modification system produces that plants are infertile, future experiments are needed to correct this problem(Kubota et al., 2012). Another possibility for genetic modifying the asparagus cultivars is the direct transfer of genes to protoplasts in order to make fertile transformed plants for future breeding(Conner & Abernethy, 1996).

The trade barriers would include prohibition and taxation of products such as the ban on forestry product exports(Bishnu P. Sharma, Shiva R. Adhikari, 2014; Nepal Ministry of Finance, 2016), the lack of electricity, limited free labour, high transportation costs and general infrastructure problems(Bishnu P. Sharma, Shiva R. Adhikari, 2014; Nepal Ministry of Finance, 2016). In conclusion the prospect of an asparagus germplasm trade is unlikely to work until the trade problems solved and the breeding methods are modified(Bishnu P. Sharma, Shiva R. Adhikari, 2014; Kubota et al., 2012).

Alternatives to this product

One of the alternatives is the use of anther tissue culture to produce super male asparagus cultivars(Regalado et al., 2016). In the wild populations of asparagus officinalis the super male form of this species makes up 2% of the population(Regalado et al., 2016). The anther tissue culture also generates plants of which 83.3% di-diploid super males and 98.6% tetra-diploid super males(Regalado et al., 2016). These super males can be bred female asparagus plants to produce all male hybrids(Regalado et al., 2016). The substances used in this tissue culture are male microspores obtained from anthers(Regalado et al., 2016). The flower buds of the asparagus cultivar were harvested spring to obtain pollen. The flower buds were placed in a 3:1 solution of 95 % ethanol and glacial acetic acid for a day kept at a temperature of 4C use. For the harvesting process the flower buds obtained using sterilized grabbers(Regalado et al., 2016). The

flower buds were cleaned by washing them with soapy water, treated with 1 % of sodium hypochlorite for15 min in a vacuum and the buds put in three washes with pure water to eliminate the sodium hypochlorite and removed with sterilized grabbers(Regalado et al., 2016). The MAE medium is then used to induce callus tissue formation using cold shock treatment on the microspore samples(Regalado et al., 2016). The callus samples made from the anther pollen are then placed in proliferation media (PM) to mass produce the tissue samples at a 80% success rate(Regalado et al., 2016). This process can be used by the breeders of Nepal to improve their own germplasm. A more complete explanation of this process can be found on the paper: Conversion of a male-specific RAPD marker into an STS marker in Asparagus officinalis L.(Regalado et al., 2016)

Another method to determine whether the plant would be to use a single step DNA extraction and a multiplex PCR method to confirm the plant samples sex(Ii et al., 2012). This method could be used alongside the anther tissue culture method or independently from this process,(Prague et al., 2012; Regalado et al., 2016).

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