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Plant Propagation and Commercial Cultivation in the Micronesian Region: Challenges and Measures for Sustainable Black Pepper Production

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Abstract

Black pepper (*Piper nigrum* L.), a flowering vine of the Piperaceae family, is valued for its dried berries called peppercorns, which are known for their health benefits and are used as a spice and seasoning. Native to the humid jungles of the Malabar Coast of Southwestern India, the plant is cultivated in the tropics worldwide. In the Micronesian region, it is gaining commercial importance as an important cash crop because of the premium price of peppercorns. However, the limited availability of disease-free black pepper seedlings and the trunks of the native tree fern (*Cyathea nigricans*) that are used as supports for black pepper vines are becoming limitations for sustainable commercial black pepper cultivation in the region. Therefore, to ensure the year-round availability of uniform, disease-free and high-quality planting material in Micronesia, an efficient micropropagation and acclimatization protocol was developed for a local, commercially important black pepper cultivar (*Piper nigrum* cv. Srilanka). Shoot apical meristems were used as explants for culture establishment. The best culture initiation was observed on Murashige and Skoog medium augmented with 5 µM 6-benzylaminopurine (BA). For further growth and subsequent multiplication, the established cultures were transferred onto 7.5 µM BA and 5 µM indole-3-acetic acid (IAA). The number of multiple shoots produced from each explant after two subcultures varied from 8 to 20. The best rooting was observed on 2 µM indole-3-butyric acid (IBA). Plantlets were acclimatized with 68% survival rate in 10 weeks. Research trials for sustainable commercial black pepper cultivation were designed and implemented, and vigorous vegetative growth was observed. To overcome the limitations of live tree-fern supports, non-living supports such as reinforced cement-concrete standards were specifically designed and used to support the vines for commercial cultivation. First harvesting was done after 12 months of planting

and data collection and analysis is underway. The outcome of this analysis will be used to provide assistance to the regional farming communities to promote sustainable commercial cultivation of black pepper in the region.

INTRODUCTION

Black pepper (*Piper nigrum* L.) is a perennial woody climbing liana. The plant is native to India, Indonesia, Malaysia, South America, and the West Indies, but is also widely cultivated in tropical regions worldwide. Black pepper is a universal table condiment used to flavor all types of cuisines worldwide. It is considered as the 'King of Spices' (Nair, 2004; Srinivasan, 2007). The spicy taste is mainly due to the presence of the compound piperine. Piperine is a pungent alkaloid (Tripathi et al., 1996) that enhances the bioavailability of various structurally and therapeutically diverse drugs (Khajuria et al., 2002). Increasingly popular modern-day uses of piperine, the active principal of black pepper, are to stimulate metabolism, aid absorption of nutrients, and boost the efficacy of drugs (Szallasi, 2005).

In most pepper-producing countries, black pepper is a smallholder's crop and many farmers depend on it for their livelihood. Cultivation varies from intensive monoculture to extensive homestead gardens. The use of reliable standards (supports) for the successful establishment of black pepper plantations is a common practice in producing countries. Standards are of two types: living and non-living. The use of non-living (dead) standards (reinforced cement concrete posts, granite pillars, and teak poles), though often resulting in higher black pepper yields, is less widely practiced by smallholders, mainly due to the high capital investment required (Dinesh et al., 2005). Non-living standards have been used in Malaysia, Vietnam, Brazil, Thailand, and Indonesia, facilitating closer spacing and higher yields (Kurien et al., 1985; Menon et al., 1982; Reddy et al., 1992).

Black pepper is considered an important cash crop in the Pacific, specifically in Micronesia. The Micronesian islands lying just above the Equator enjoy a tropical climate with relatively even, warm temperatures throughout the year. The Federated States of Micronesia (FSM) is made up of 607 small islands spread over a million square miles of the Western Pacific Ocean with a total land area of only about 271 square miles. Rainfall is generally plentiful, and Pohnpei, the capital state, is reputedly one of the wettest places on Earth, with up to 330 inches of rain per year. Nevertheless, drought conditions do occur periodically throughout FSM, especially when the El Niño condition moves into the Western Pacific. During these times, groundwater supplies have dwindled to emergency proportions. Tropical typhoons constitute an annual threat, particularly to the low-lying atolls of FSM (Government of the Federated States of Micronesia, 2014).

Agriculture is an important industry and can greatly help with economic development and growth, and in bringing food self-sufficiency to Micronesia. However, current agricultural programs in the country are mostly on a subsistence level and economic development is largely dependent on the outside world. Serious damage caused by natural calamities, such as wave surges, saltwater flooding, and drought, continually pose challenges for the local farmers. Moreover, a lack of technical know-how and changing lifestyle and food habits of the islanders are causing an increase in the consumption of imported foodstuff, leading to an overall decline in local agricultural production.

One recent example of such decline in agricultural production is the ceasing of local production of black pepper in Pohnpei, Micronesia. Black pepper from Pohnpei, Micronesia, is regarded as a relatively rare commodity of exceptionally high quality. The "Pohnpei Pepper", a pepper product that was unique to Pohnpei was marketed successfully from Pohnpei for a short time. The product was admittedly a high-end, niche product without a large volume of sales, but the potential of the product was barely tapped before its production ceased (Cheshire, 2003). Therefore, to promote sustainable black pepper cultivation practices in Micronesia, a project was developed to support local farmers and enhance agricultural productivity of black pepper in the region.

Based on the inputs of the stakeholders, farmers, agricultural professionals, and direct observations during black pepper farm visits, the project team identified the following issues that have caused serious decline in black pepper production in the Micronesian region:

- Non-availability of elite and disease-free seedlings;
- Limited traditional tree fern supports (standards).
- Poor soil fertility management and fertilizer applications.
- Occurrence of pests, diseases, and nutrient deficiencies.
- Shortage of trained agricultural professionals.
- Inadequate knowledge for fast propagation of cash crops.
- Limited skills for commercial production and basic crop management.
- Insufficient storage and processing facilities.
- Stiff competition between local and imported products.

The non-availability of disease-free and elite seedlings is a major bottleneck in quality black pepper production in the region. Micronesia is a small islands state; therefore, quarantine measures are very strict, and the entry of any planting material is strictly prohibited. Considering the difficulty in maintaining disease-free parental stocks in the tropics, meristem culture is increasingly being appreciated as a potential means of germplasm preservation and for the production of elite and disease-free planting materials on a mass scale.

Plant biotechnology is a powerful tool. Appropriate and skillful use of biotechnological approaches, such as in vitro multiplication, could help in successful multiplication of the elite and disease-free seedlings of cash crops and provide a means for germplasm conservation in an inexpensive way. In vitro multiplication is the best way to multiply the planting material of vegetatively propagated crops in bulk and produce disease-free, elite seedlings throughout the year.

The project aims to address some of the prime agricultural issues affecting the commercial black pepper production in Micronesia. It is specifically designed to develop micropropagation and nursery management systems in the region. The objectives of the project include: produce elite black pepper seedlings in bulk quantities to ensure the year-round availability of identical, disease-free and high-quality planting material; find alternatives for tree fern supports; determine appropriate fertilizer type and rates; and publish a commercial black pepper cultivation guide appropriate for Micronesia. Adoption of new practices, such as micropropagation of black pepper for improvement and enhanced productivity, will ultimately help in reviving the local pepper industry. The following sections describe the health benefits, in vitro multiplication, and commercial cultivation of black pepper.

IN VITRO MULTIPLICATION OF BLACK PEPPER

Plant Material

To establish cultures for in vitro multiplication of black pepper (*Piper nigrum* cv. Srilanka), 10- to 15-cm-long vines with shoot apex were collected in the morning from visually healthy, one-year-old plants that were growing in farmers' fields. The collected vines were kept in a greenhouse in which 100% relative humidity was maintained. Explants from these vines were obtained within 2 to 4 hours of their collection.

To obtain explants, vines were further trimmed to 4-cm-long tips containing the shoot apex. These trimmed vine tips were thoroughly washed with running tap water and were surface sterilized by immersion in 50% (v/v) ethanol for 10 minutes, which was followed by a treatment with 2% (v/v) sodium hypochlorite solution with 5 drops of Tween 20 for 5 minutes. Sterilized vine tips were then rinsed 5 times with sterile distilled water and were kept immersed in the solution until shoot apical meristem explants (including surrounding base tissue of 0.5 × 0.5 cm size) were excised for in vitro culture establishment.

Culture Medium

Murashige and Skoog (1962) medium (MS) was used in this study as a basal medium. All media contained 0.8% agar and 3% sucrose. The pH was adjusted to 5.8 prior to autoclaving. Different concentrations and combinations of cytokinins and/or auxins were used to augment the media for culture establishment, multiplication, and rooting.

Micropropagation

Shoot apical meristem explants were inoculated on MS medium without any growth regulator and supplemented with 50 µM cupric sulfate pentahydrate (CSP). Excellent establishment of black pepper cultures was observed in 6 weeks. Adding CSP in the media reduced the rate of fungal and bacterial contamination rate to less than 1%. Micropropagation of black pepper occurred in three distinct phases: 1) shoot initiation phase (SIP); 2) shoot multiplication phase (SMP); and 3) shoot elongation phase (SEP) (Figure 1a, 1b, 1c). To induce shoot initiation, the established cultures were transferred onto MS medium augmented with 5 µM 6-benzylaminopurine (BA) on which they were kept for 6 weeks and a passage (additional transfer) was given for another 6 weeks. After 12 weeks of incubation on SIP media, 100% shoot initiation was observed in all cultures. SIP cultures were then transferred onto MS medium augmented with 7.5 µM BA and 5 µM indole-3-acetic acid (IAA) for 6 weeks to induce shoot multiplication, and two passages at 6 weeks each were given for subsequent multiplication during SMP. Multiple shoots induced during SMP were then transferred onto MS medium without any growth regulators for 6 weeks, and two passages were given at 6 weeks each for further growth and shoot elongation during SEP. The total incubation time for culture establishment and all three phases was 56 weeks, with the duration of culture establishment being 6 weeks long, SIP being 12 weeks long, SMP being 18 weeks long, and SEP being 18 weeks long. The number of elongated shoots of 7 to 9 cm size produced from each explant after two subcultures varied from 8 to 20. Every 6 weeks, data were recorded, and each experiment was replicated three times with 25 explants per replication. A photoperiod of 16 hours with 40 µmol m^{-2} s^{-1} light intensity along with 24° C day and 22° C night temperatures were maintained during all phases of micropropagation.

Rooting

MS medium augmented with 2µM indole-3-butyric acid (IBA) proved best for inducing rooting of multiple shoots obtained through SEP (Figure 1c). Fully elongated shoots of 7 to 9 cm in height were transferred onto the rooting medium in groups of 20 to 25 shoots per culture (Figure 1c). After 4 weeks on rooting medium (Figure 1d), the percentage of rooting, number of roots per shoot, and root length were recorded. Each experiment was replicated three times with 100 cultures per replication.

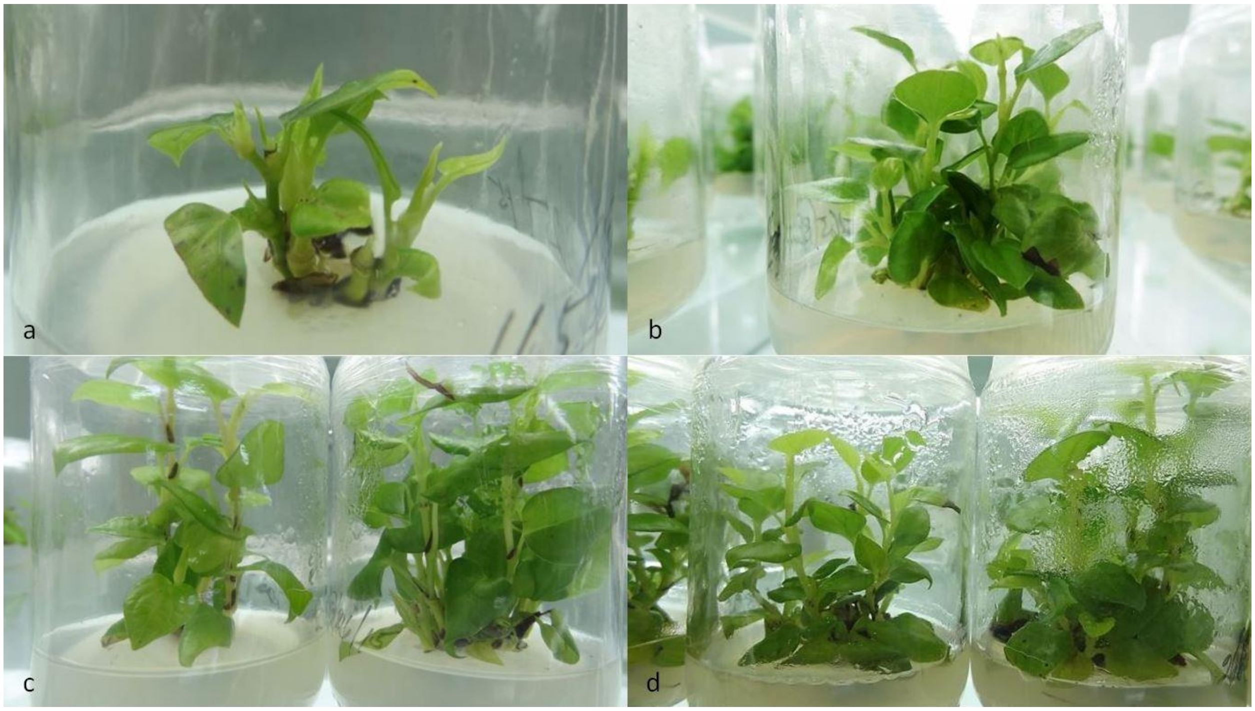

Figure 1. Black pepper culture establishment (a), black pepper multiplication (b), rooting of black pepper multiple shoots (c), and complete black pepper plantlets (d).

Acclimation

Complete plantlets with 8- to 10-cm-long roots were transferred into 10-cm pots containing a sterilized soil:vermiculate (1:1, v/v) mixture. These potted plantlets were kept in the greenhouse for the first 6 weeks and then transferred into the screen house for the next 12 to 18 weeks. The screen house was covered with 60% green, knitted shade cloth and the temperature was maintained between 26^{o}C and 28^{o}C with 50% to 55% humidity. During these phases of acclimatization, the plants were irrigated once every 2 days with tap water and once per week with a one-fourth strength solution of MS basal salts. The survival rate of the plants was recorded after 10 weeks and a 68% survival rate was achieved. Each experiment was replicated three times with 100 plantlets per replication. After 12 to 18 weeks of ex vitro growth in the screen house, completely acclimatized plants were transferred to the nursery where they were kept until transfer to the field.

Figure 2. Acclimatization of black pepper plantlets (a), disease-free acclimatized black pepper seedlings in nursery (b), raised beds for proper water drainage and durable reinforced cement-concrete standards to support black pepper vines (c), and planting holes 1.5 feet across and 1.5 feet deep (d).

Statistical Analysis

Each experiment was replicated three times. A one-way analysis of variance was used to determine the level of significance between experimental treatments. Statistical significance of the results was determined using the least significant difference (LSD) test at α=5%.

BLACK PEPPER: COMMERCIAL CULTIVATION

Black pepper is valued for its dried berries, called peppercorns, which are used as a spice and for medicinal purposes. Native to the humid jungles of the Malabar Coast of Southwestern India, the plant is cultivated in the tropics worldwide. In Micronesia, it is gaining commercial importance as an important cash crop because of premium price. Traditionally, the trunks of two cultivars of large native tree fern (*Cyathea nigricans*) are used as supports for black pepper cultivation. However, short lifespan of these tree ferns, along with the rapid decline in their population due to increasing use of tree trunks for construction, is becoming a limitation for commercial black pepper cultivation in the region.

An in vitro multiplication protocol for locally preferred and commercially important black pepper cultivar *Piper nigrum* cv. Srilanka was developed and utilized for the multiplication and production of elite, uniform, and diseases-free black pepper plantlets in Micronesia. An efficient nursery management system was also standardized for the acclimatization of hundreds of plantlets into uniform and disease-free seedlings for sustainable commercial cultivation.

In Micronesia, the trunks of tree fern (*Cyathea nigricans*) are traditionally used as living supports for commercial black pepper vines. These large, native tree ferns are important sources of wood and are used for traditional house construction and as supports for commercial black pepper cultivation. Of the two cultivars of tree ferns that are traditionally recognized, one produces a red staining juice and is preferred over the other cultivar, which produces a greyish juice. The increasing construction in Pohnpei, along with the short life span of the desired tree ferns, has resulted in a drastic reduction in their lowland population. With newer roads now providing access to several inland locations, the upland populations of tree ferns are also threatened (University of Hawaii, 2014).

Considering the increasing demand for commercial black pepper cultivation and the extremely limited availability of traditional tree fern supports, non-living supports such as reinforced cement-concrete standards have been specifically designed and constructed at the pilot site to support the vines of fully acclimatized black pepper plants in the field. Standards of reinforced cement-concrete (6-inch length and 6-inch width in an octagonal shape and 13- to 15-foot height) were constructed and used as a support for each plant. In addition, raised beds, which ensure perfect water drainage, were used for the establishment of black pepper commercial plantations. To provide perfect nutrition and maintain these plantations, organic fertilizers, along with organic mulching and automatic fertilizer injectors, were used for soil amendment (Figure 3).

Figure 3. Soil amended with organic fertilizers for black pepper planting (a); liquid fertilizer application to each plant at regular intervals by automatic fertilizer injector (b, c), and healthy and vigorous black pepper vine vegetative growth (d).

This project is integrating and employing the latest tools and technologies, such as plant biotechnology, horticulture, microbiology, plant physiology, and plant pathology, for the sustainable, climate-smart and organic commercial cultivation of black pepper in Micronesia. The project team is utilizing plant biotechnological techniques, such as in vitro multiplication, for uniform black pepper plantlet production and greenhouse acclimatization of multiplied black pepper plantlets for elite, disease-free seedling production. The team is also using automatic fertilizer injectors for uniform fertilizer application, organic fertilizers to provide essential nutrients and maintain beneficial soil microorganisms, and appropriate site-specific and climate-smart horticultural, plant physiological, and integrated pest and disease management practices.

Climatic Conditions

Black pepper originates from tropical, warm, humid latitudes, where temperatures of 77^{o}F and 80 to 120 inches annual rainfall predominate. Evenly distributed rainfall is ideal. Supplemental irrigation is necessary in dry, low-rainfall areas. Due to its tropical climate and adequate rainfall, pepper can be grown throughout the year in Micronesia.

Soil Characteristics

Black pepper can be grown on a wide range of soil types, but best results are obtained on deep, well-drained soils with good water-holding capacity. The best soil characteristics are sandy clay loam to clay loam with adequate essential plant nutrients and high organic content. Suitable soil pH is between 5.0 to 6.5. A slope not exceeding 10^{o} to 15^{o} is recommended for better soil conservation, easier harvesting, and farm management.

Field Preparation

Soil preparation for black pepper is similar to that for most dryland crops, such as corn. Existing vegetation is turned under with a moldboard or disc plow, or by spading. Most soils benefit from adding compost at this stage. During cultivation, phosphate fertilizer can also be added if required. After turning, leave the soil for a few days to allow for decomposition, and then break soil clods by harrowing or rotovating (or with a hoe or rake in small gardens). After the soil has been pulverized, the surface should be smoothed in preparation for black pepper planting. Black pepper can be planted on ridges, in furrows, or on flat ground.

Preparation of Planting Materials

Traditionally, black pepper has been propagated through cuttings that are prepared from stock plants. The cuttings consist of the upper 5 to 7 nodal segments. Selected planting materials should come from varieties that are disease- and pest-resistant, vigorous, and high yielding, with good productivity with respect to the final product. In recent years, owing to the advantages of disease-free planting material and uniformity in growth and higher yields, the use of tissue cultured plantlets as planting material for black pepper has become increasingly popular among farmers.

Supports and Planting

In Micronesia, the trunks of the tree fern (*Cyathea nigricans*) are traditionally used as living supports for commercial black pepper vines. Considering the extremely limited availability of tree fern supports and their very short lifespan, non-living supports such as reinforced cement-concrete supports are a good alternative. Supports should be planted well before planting black pepper at a depth of 2 to 3 feet. The planting holes should have a depth of 1.5 feet and a radius of at least 1.5 feet from the support. Prior to planting, the soil should be amended adequately with organic fertilizers, such as compost. Disease-free seedlings should be planted in prepared holes at the onset of a rainy day or in the evening. Young vines should be tied loosely to the support and shaded with suitable plant material.

Considering the frequent and heavy rains and poor drainage in the Micronesian region, planting black pepper seedlings in rows on raised beds is recommended (Figure 4a). The plants should be spaced in the rows at 8 feet apart with a 10-feet-wide alley maintained between rows.

Figure 4. Healthy and vigorous black pepper vines: One year old after planting (a); black pepper vine pruning (b); black pepper flowering (c); and ready-to -harvest drupes of black pepper (d).

Pruning

A couple rounds of pruning should be carried out during the vegetative phase of vine growth. Initial pruning of terminal shoots is done 4 to 6 months after planting. The next pruning is done when the vines are about 1 year old (Figure 4b), and the last pruning is done when the terminal shoots have reached the top of the standards.

Irrigation

Often grown in areas with high rainfall, black pepper is generally a rain-fed crop. Black pepper plantations do not require irrigation under normal conditions, except perhaps during the initial establishment period or in drought-prone areas. The plantations should not be allowed to become waterlogged for any extended length of time. For best results, maintain soil moisture at or near field capacity (moist but fully drained) throughout the growing period.

Fertilizer Application

Soils should be analyzed for nutrition status to determine nutrient requirements for growth and productivity of black pepper vines. In a tropical climate, it is better to apply small quantities of fertilizer often, rather than to add a large quantity in one treatment. This makes the fertilizer application more profitable and prevents too much rapid growth. Black pepper requires good soil fertility. In the first year, organic fertilizers such as compost may be applied at the rate of 4 to 6 pounds, along with 0.25 pounds of inorganic fertilizer, such as 12:2:14 NPK plus micronutrients every 3 months. In the second year, organic fertilizers may be applied at a rate of 8 to 10 pounds, along with 0.50 pounds of inorganic fertilizer, such as 16:16:16 NPK, plus micro-elements every 3 months. In the third year and onwards, organic fertilizers may be applied at a rate of 10 to 12 pounds, along with 1.0 to 1.5 pounds of inorganic fertilizer, such as 12:12:17 NPK plus micro-elements every 3 months.

To apply compost or organic fertilizers, scrape the soil surface around the circumference of the canopy. Apply the fertilizer along with the organic fertilizers at the recommended rate and then cover it with soil taken from the inter-spaces. Ensure sufficient moisture availability during fertilizer application.

Weed Control

Black pepper is susceptible to weed competition, especially during the first 8 to 12 months after planting when the leaf canopy is being formed. During this time, control weeds by hand-pulling or cultivating with a hoe. After the crop has attained the maximum vegetative stage, the lush foliage will shade out weed growth and cultivation for weed control should be minimized to avoid injuring the roots. When necessary, limited weeding by hand may be done in the inter-spaces and around the base of the vine.

Insect Pests and Diseases

Nematode infestation by *Meloidogyne* spp. causes the main problem in conventional pepper cultivation. Soil-borne fungi are the most significant cause of disease to black pepper. They possess a wide spectrum of hosts and can affect practically all of the crop types. Therefore, carry out constant and frequent scrutiny to identify any incidence of disease or pest at an early stage and take immediate action to control them. Integrated pest and disease management principles need to be applied at all stages to maximize productivity and minimize crop loss. Phytosanitary measures, such as physical removal of pests, affected plant parts, infected plants (virus-infected plants, severely disease-infected or pest-infested plants, including plants affected by *Phytophthora* spp. or slow decline or yellow wilt) are important to control these incidents.

Organic plant products and biocontrol agents, such as neem oil, neem cake, hot chili pepper solutions, and recommended predators for insect pest control may be used. Agrochemicals for control of pests and diseases may be used only when all other measures have been exhausted. Chemicals used should comply with all state regulations. Application of chemicals should follow recommended practices and chemicals should be applied only under the supervision of qualified professionals.

Harvesting

Each flower on the pendulous inflorescence of black pepper can develop into a single-seeded drupe (Figure 4c). Drupes that are almost mature with all green berries can be picked to process as green pepper. Drupes on an inflorescence with one or two berries beginning to turn yellow can be picked to process into black pepper (Figure 4d). To process into white pepper, drupes should be fully mature, with one or two ripe yellow-orange berries on each drupe. Drupes should be picked selectively and harvesting rounds should be carried out frequently throughout the year. Harvested drupes of pepper should be handled hygienically, collected, and transported in clean and closed baskets for processing into peppercorns (International Pepper Community, 2008).

Processing, Drying, and Storage

To ensure high quality, threshing of green pepper berries from the drupes is done manually in Micronesia. Separated green pepper should be washed in clean water to remove field dirt, insects, or other contaminants that may be present. Washed, cleaned pepper should be soaked for 1 to 2 minutes in water at 194°F to eliminate contaminants. Soaking in hot water would also facilitate drying and improve the appearance of the dried peppercorns. In Micronesia, solar dryers and electric dehydrators are used because of frequent rain and extremely high relative humidity. Black peppercorns should be dried to a moisture level of 10% for long storage. To avoid the loss of volatiles in peppercorns, drying must not be done at temperatures above 131^{o}F.

Texture and Color

Different harvesting times and processing techniques can result in various colors and textures of peppercorns (Naturland, 2001). For green pepper, green peppercorns are produced when almost-mature green berries are harvested, processed, and conserved in brine (salt water, vinegar, and citric acid). For black pepper, black peppercorns are produced when mature green berries are harvested, processed, and dried (Figure 5a, 5b, 5d). For white pepper, white peppercorns are produced when ripe yellow-orange berries are harvested, processed and dried (Figure 5c).

Figure 5. Harvested drupes of black pepper (a), processing of black pepper (b), processed white peppercorns (c), and processed black peppercorns (d).

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