Complementary conservation of coconuts

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Introduction

The main objective in any plant genetic resources (PGR) conservation programme is to maintain the highest possible level of genetic variability present across the genepool of a given species or crop both in its natural range and in a germplasm collection. The importance of conserving genetic variability or diversity is well recognized and such defense mechanisms need to be introduced into modern cultivars to make them sustainable (Martin *et al.* 1991; Chang 1994; Kannenberg and Falk 1995).

Countries that are signatories to the Convention of Biological Diversity endowed with significant amount of genetic and species diversity have a responsibility to the world at large to conserve them and make them available for use (Ramanatha Rao 1999). It is generally recognized that the two approaches of conservation, *ex situ* and *in situ*, are both important in the conservation and use of genetic diversity and should be regarded as complementary to each other (Maxted *et al*. 1997; Dulloo *et al.* 1998; Ramanatha Rao 1998; Engels and Wood 1999). The ultimate purpose of germplasm conservation is use and, consequently, any conservation strategy should include mechanisms that will ensure access to the germplasm by relevant stakeholders. Other important issues that must be addressed in a conservation strategy include issues related to policy and legal frameworks, documentation, socioeconomic aspects, infrastructure and networks. Since needs of users and technologies may change over time influencing the ways in which genetic resources are conserved and used in future and hence, should be taken into consideration when designing a conservation strategy. At an in house meeting organized by IPGRI in 2002, a complementary conservation strategy was defined as "the combination of different conservation actions, which together lead to an optimum sustainable use of genetic diversity existing in a target genepool, in the present and future."

A conservation strategy for coconut has been discussed in the past (Ramanatha Rao and Engelmann 2000; Ramanatha Rao *et al.* 1998) and the current status of the various conservation methods available for coconuts are described in this chapter**.** This paper discusses the constraints and advantages of these methods, the elements for a complementary conservation strategy and attempts to provide a framework from which a working strategy for conservation and use of coconut germplasm could be taken forward.

Methods for conserving coconut germplasm

As noted earlier, PGR are commonly conserved using *ex situ* or *in situ* approaches. *Ex situ* refers to their conservation outside their natural habitat in facilities such as in seed banks, field genebanks, *in vitro* collections, botanic gardens, with germplasm conserved in the form of plants, seeds, pollen, tissues, cells or DNA. In contrast, *in situ* conservation is conserving germplasm in the natural habitat where the target species is found, and in habitats such as farms and home gardens, where the species have developed their distinctive properties as a result of long-term selection by humans. The latter applies particularly to cultivated plants and their cultivars, landraces and weedy forms. Generally, there are three categories of *in situ* reserves: namely, those which maintain optimum conditions such as national parks and nature reserves, those which allow a range of economic activities by indigenous people as in extractive reserves national forests and Biosphere reserves, and a third category where local people act as custodians for the traditional varieties and selections contained in home gardens and farms (Damania 1996). Furthermore, IUCN (1994) classifies protected areas into six categories according to broad management objectives.

The previous chapters have described in detail the current status of conservation techniques coconut germplasm, an analysis of which could help in developing a complementary conservation strategy for coconuts. Table 1 examines the feasibility of different techniques, while Table 2 summarizes the constraints and advantages with regard to each of these methods. These two tables provide a comparative framework on which a complementary conservation strategy for coconuts could be based. It is important to emphasize that the information in Table 1 is based on our current knowledge, which could rapidly change in the near future, due to progress made in the development of the conservation methodologies. The biological characteristics of coconut and their compatibility with different options available are briefly discussed below. It is important to underline a fact at this point that when several options are combined to bring about a complementary strategy, we also bring along their advantages and disadvantages with the expectation that a synergistic effect is achieved.

The options for conserving coconuts are dependent on the biological characteristics of the whole plant and its component organs and tissues, as well as on the state of the technology as applied to coconuts. Coconut, a perennial palm, with exception of most of the Dwarfs, is an outbreeding species. It bears large size seeds that exhibit recalcitrant storage behaviour, rendering seed conservation not possible. Being a perennial crop, coconuts can be conserved *ex situ* as live plants in field genebanks or botanic gardens or *in situ* either on farm or in home gardens or on remote islands and atolls. Botanic gardens have limited capacity to conserve a broad range of genetic diversity due to the low number of plants that they can maintain. Field genebanks (attached to a coconut improvement organization) have been the preferred mode of coconut conservation to date, as they can be integrated into institutions and do not require highly technically skilled workers (Ramanatha Rao *et al.* 1998). However, field collections have some major disadvantages (Table 2). Coconuts are generally outbreeding, especially the Tall types, and requires wither spatial isolation or assisted-pollination. There are still some important research questions to be addressed in regard to collection management such as minimum number of trees needed to maintain representative genetic diversity, field plot techniques for characterization and evaluation and economics of collection maintenance. For details on field genebanks and the ICG, see related articles in this chapter.

On-farm conservation, where traditional crop cultivars or landraces and/or farming systems by farmers within traditional agricultural systems are maintained (Hodgkin *et al.* 1993; Jarvis 1999), has been gaining importance over the last decade. For coconuts, this method is particularly advantageous since most of the stands in South and Southeast Asia are in more or less intensively managed areas. For effective on-farm conservation, knowledge on the effects of farmers' practices on the extent and distribution of genetic diversity information on history of coconut cultivation and indigenous knowledge and actual genetic diversity measurements may be required. It is now possible to monitor and estimate genetic diversity using molecular markers for coconuts (Foale 1992; Ashburner and Rhode 1994; Lebrun *et al.* 1998; Mpunami *et al.* 1998; Perera *et al.* 1999). People's participation and cooperation among local people, researchers and conservationists and non-governmental organizations (NGOs), are essential ingredients of success for the sustainability of on-farm conservation efforts. Furthermore, any *in situ* conservation programme must benefit the local communities. Establishment of areas of intensive management or high yielding plantations would assist long-term sustainability of *in situ* conservation programmes. This is not to replace, but to bring a balance between high-yielding types for purely commercial purpose and landraces to satisfy all the personal and social needs of farmers. Such a balance is essential to promote conservation of landraces in the absence of any specific additional benefits to growers. This can attract commercial and private agencies to be partners in on-farm conservation efforts and can lead to much wanted linkages between public, community and private sectors in PGR conservation. For naturally occurring coconuts palms other forms of *in situ* conservation such as island reserves, biosphere reserves may have very important complementary value in conserving unique diversity as for example populations isolated on small uninhabited islands and atolls (Ramanatha Rao *et al.* 2000). For more detailed description of on-farm conservation, see related articles in this chapter.

Progress achieved in recent years in *in vitro* culture and cryopreservation as potential methods for conserving coconut germplasm augurs well for the future. Research on the development of such techniques has been performed with zygotic embryos, somatic embryos, pollen, apices and DNA material (Assy-Bah and Engelmann 1993). *In vitro* culture of zygotic embryos has been significantly improved and is now operational in an increasing number of laboratories (Engelmann *et al.* 2002). An efficient cryopreservation protocol has been developed for zygotic embryos (Assy-Bah and Engelmann 1992), which needs to be refined and tested on a range of ecotypes before becoming fully operational. Somatic embryos cannot be used for germplasm conservation since *in vitro* propagation of coconut using somatic embryogenesis is not yet functional. Cryopreservation of apices ('plumules') sampled from zygotic embryos is also possible (Hornung *et al*. 2001; Malaurie *et al.* 2002) but regeneration of whole plants from such explants is difficult. At the moment, plumules are of no use for germplasm conservation except possibly in case it would be proven that diseases (MLOs) can be transmitted through the embryo. DNA material can be cryopreserved easily and can be of great value in genetic diversity studies. However, regeneration into whole plants is problematic, if at all possible. For more details, see related articles in this chapter.

Conservation of coconut pollen is an additional option. Pollen can be dried and stored under vacuum for a short period of time (2-6 months) in a domestic deep freezer (Rognon and de Nucé de Lamothe 1978). Freeze-drying experiments showed no viability loss after 3 and 6 months (Whitehead 1966; Benard 1973) of storage at room temperature. Coconut pollen is highly tolerant to desiccation and preliminary experiments have demonstrated that coconut pollen could be successfully cryopreserved (Dr. Assy-Bah, unpublished results). Long-term storage of coconut pollen under cryopreservation would represent an important additional technique for genetic resources conservation, by allowing conservation of genes. However, additional research is still needed to further develop and refine a cryopreservation protocol.

Considerations for complementary conservation strategy

The knowledge of the biological characteristics of coconuts and how they can be conserved, as discussed above, is just one of the many elements for developing a sustainable complementary conservation strategy. This section discusses some other important elements, which need to be taken in to consideration.

Conservation objective

The most central element for developing a strategy is to define precisely what the objectives are. In this case, the general objective is to conserve and utilize maximum coconut genetic diversity. However, there would be other minor objectives for the establishment of a coconut genebank such as for immediate utilization, conservation for the long term, focusing on characterization and evaluation, etc. Strategy applied will also depend on what one would want to conserve, i.e. genes or genotypes. The strategy will be very different if the objective is to completely stop the evolutionary processes (e.g. cryopreservation) or in case the evolutionary processes need to be maintained (as in *in situ* conservation). Thus, if the promotion of conservation of landraces becomes the main objective, conservation on farm becomes the choice strategy for coconut, which also provides an opportunity for coconut to evolve under natural and farmer-imposed conditions. However, there is a need to accumulate more evidence on the role of farmer selection in a perennial crop like coconut. At the same time, with many farmers interested in increasing the productivity of coconut and income generation, breeding for higher yields and multiple uses becomes priority and hence *ex situ* conservation in field genebanks, which enhance the access to diversity by the coconut improvement scientists, becomes the choice for conserving and using maximum genetic diversity.

Genetic diversity

The major objective of any conservation effort, especially the one for long-term, is the conservation of maximum genetic diversity in a crop gene pool and this is true for coconut as well. Hence, the factors that contribute to the maximization of genetic diversity in a coconut collection (only infraspecific diversity in the case of coconut) have significant bearing on the balance of options chosen for inclusion in a conservation strategy. Coconut belongs to a monotypic genus and hence all its genetic diversity is in one species, i.e. *Cocos nucifera.* The diversity in coconut is mainly in the different ecotypes/landraces, i.e. conservation of genotypes and, consequently, using the field genebank allows conservation of most genetic diversity in the gene pool. Since very little information is available on the extent and distribution of coconut genetic diversity within and between populations and the genetics of useful traits, probability theory and random sampling (at times modified to include some level of bias for elite material, which is generally the norm for horticultural and perennial species) and larger populations are used to locate and conserve the desired level of genetic diversity. Under the ADB-funded project of COGENT, 28 countries have collected and conserved coconut germplasm in national field genebanks and a multi-site International Coconut Genebank (ICG) has been established, which makes the access to genetic diversity easy (see Batugal and Kanniah in this chapter). At the same time, COGENT also recognizes the limitations of the field genebanks. By using on-farm conservation, it is possible to conserve more diversity, especially that diversity which is directly useful to farmers. To do proper on-farm conservation, essential information on the extent and distribution of genetic diversity on farms is being generated. The limited observations to date have shown that very few farmers seem to pay any special attention to phenotypic and other differences in coconut types that they grow. Most often coconuts are just planted and little attention is paid later on. Hence, the so called indigenous knowledge on coconuts seems to be limited. Nevertheless, there are some who recognize this well, and hence should be targets for on-farm conservation efforts. Field genebanks require a substantial number of individual genotypes to be an effective conservation measure. Thus, extensive network of farm sites will be able to complement conservation of genetic diversity in coconut.

Stakeholders

Conservation of any genepool is not just a responsibility of an organization or individual. Several interested organizations and individuals are involved, including those who were responsible for the generation of the variability in the first place. Thus, in the case of coconut, the interests of small coconut farmers, organizations interested in their welfare and coconut research organizations/scientists and at the end the consumers, etc., need to be considered. For example, coconut farmers for whom coconut growing is a way of life and in some instances, growing the specific landraces or ecotypes, on-farm conservation takes precedence over the other approaches. This needs to be strengthened and complemented by other stakeholders who can play an important role in conserving that part of coconut diversity that might not be conserved on-farm due to reasons such as genetic erosion and utilization, using other complementary approaches such as conservation in field genebanks.

Infrastructure

The infrastructure needed and their availability determine the option to be chosen. Hence, the infrastructure needs for each of the option and their availability and resources required needs to be documented and analyzed. For example, the establishment of a field genebank for coconut genetic resources requires land, labour, good agronomy, facilities for exchange of germplasm, well-trained staff, etc. *In vitro* culture and cryopreservation would also require specific infrastructure and highly trained skilled staff. For on-farm conservation, identification of sites with high levels of genetic diversity, committed community-based organizations, staff skilled in working along with partners and farmers, access to conservation sites, monitoring mechanisms etc, have to be in place. Once such baseline information is available, then it should be possible to determine which approach will be used to particular part of coconut genetic diversity.

Socioeconomic aspects

The social considerations probably are more important in implementing on-farm conservation and less so while establishing *ex situ* conservation facilities. However, the economic aspect would be a key determinant in what methods are utilized. While planning for the former, several issues related to socioeconomics of coconut farming, indigenous knowledge, community participation, etc. have to be considered that make the on-farm conservation sustainable. Such considerations also make germplasm conserved on farm accessible for use by the farmers and communities as well as national agricultural research systems. Generally speaking, in the countries that are interested in conservation and use of coconut, the cultivation of coconut is not greatly threatened and will continue in the end. This consideration is important as establishing either *ex situ* or *in situ* conservation programmes are expensive and must be compatible with national objectives. Therefore, it is important to allow the increase of genetic diversity that is actually being planted by farmers to the extent possible. In this respect, a close cooperation between *in situ* and *ex situ* efforts is critically important.

Network

Any complementary conservation efforts for coconut at the national level have to be multidisciplinary and multi-stakeholder driven in order to conserve maximum diversity. Thus, an in-country network consisting of interested individuals, organizations (both public and non-governmental) and farmers is required. Similarly, developing a complementary conservation strategy at an international level requires coordination and collaboration among interested countries, as demonstrated by the International Coconut Genetic Resources Network (COGENT), as the genetic diversity that needs to be conserved is spread across borders.

COGENT has been able to complement the establishment of ICGs with efforts at community level that lead to on-farm conservation of coconut genetic resources. For example, the efforts to promote the cultivation of identified elite germplasm (landraces) from the genebank at sites where poverty reduction work are underway in Bangladesh, India, the Philippines, etc. This will ensure sustained conservation of landraces and at the same time benefit the poor coconut farmers.

Costs and risks

The options in any complementary conservation strategy need to be weighed against each other keeping in mind the relative costs, benefits and risks. With currently available methods, it has been generally agreed that the establishment of *ex situ* genebanks is relatively cost-effective and less risky (Pardey *et al.* 1999). However, using this one method, it would not be possible to conserve all the coconut genetic diversity that might be required in the future, especially when the number of accessions that could be maintained and managed in a field genebank is finite. Hence, a complementation by on-farm conservation of the material that would be difficult to bring to genebank becomes economical. *In situ* conservation option needs to be incorporated into the strategy. Such efforts also promote conservation through use. In addition, the analysis of the genetic diversity of coconut has shown that significant genetic diversity might exist in remote areas and atolls, collecting of which could be very expensive. One could argue that the germplasm located at these sites are relatively safe except for unfavourable climatic changes (e.g. sea level rise) which may be a risk and has to be considered. If resources are available, efforts should be made to collect and secure them in *ex situ* collections.

Policy/Legal issues

Without any doubt, for any conservation approach to be in place, much depends on the type of legal arrangements that can be put in place for transfer and access to genetic material and for sharing of benefits arising out of their use. In many countries, there may not be specific laws that prevent or promote the conservation of coconut genetic resources, but policies in a country could influence the importance accorded to such an effort. Thus, before venturing to establish a conservation strategy for coconut, it is important to check on the priority accorded to coconut at national level. For example, in most countries in the Asia Pacific, high priority is accorded to this crop and hence the efforts on its conservation and use are generally in line with the national policies. As noted earlier, conservation is mainly to make and keep the genetic resources accessible for use by users (researchers, farmers etc.). Hence the policies that promote the accessibility and transfer of material and 'information' are important for successful implementation of the different types of conservation. For example, if the national laws are very strict about collecting and using the material from farmers (as in the case of Philippines), conservation on farm may be the better option, especially for the new diversity. To establish a regional or international genebank, it is important that the partner countries policies do not hinder the exchange of coconut genetic resources, as exemplified by the agreement of participating countries in the establishment of the ICG (Ramanatha Rao and Batugal 1998).

Framework for complementary conservation strategy of coconut germplasm

It is evident from the above discussions that the options for conserving coconuts germplasm are rather limited (Table 1). The current practice, as already noted, is the use of field genebanks. On-farm conservation appears to have a great potential for such a perennial species as coconut. The perenniality, however, is also a constraint, as the information required for scientifically sound on-farm conservation would be limited. This is mainly because the information on farmers' practices in terms of selection and genetic diversity is limited since the crop's life might span over a couple of lifetimes of its growers. At present, *in vitro* collecting and *in vitro* culture of zygotic embryos that also facilitate movement of germplasm (phytosanitary aspects and cost) are fully operational. Cryopreservation, which ensures safe and cost-effective long-term storage, is expected to be operational soon after minor improvements to the existing protocol. The establishment of cryopreserved collections could be envisaged on a regional basis (e.g. one cryopreserved collection linked with each ICG site) or even on a global basis (one or two cryopreserved collections at sites agreed by COGENT partners) as a measure of long-term backup.

The balance between the different methods employed for coconuts would depend on many factors such as the intended use of the conserved germplasm, the method of maximizing the diversity of coconuts, the available infrastructure and human resources, space availability, accessibility and so on. Based on these elements and on the state of knowledge and the options available to date, a framework for complementary conservation strategy can now be developed. It is not envisaged here to develop a full strategy for coconut, but rather to propose a framework and the elements as how such a strategy could be developed at different levels: national, regional or international.

The framework can be seen as a series of steps (Figure 1). At each step information is gathered, specific actions taken and/or decision made.

The first step would be to organize the stakeholders into a network, as has happened in COGENT. This should be facilitated by a lead agency to enable its creation and be established with a steering committee composed of representatives of the various stakeholders. This would then be the decision making body to develop the strategy and take the decision on its content and implementation. The stakeholders would then be responsible to define objectives and sub-objectives according to its mandate. This would for example in the case of coconuts be to conserve and utilize the maximum genetic diversity in *Cocos nucifera*. A number of sub-objectives could also be elaborated such as the long-term conservation of coconut germplasm, conservation of specific ecotypes or characterization of germplasm, as mentioned earlier.

For each specific objective, the conservation options available should then be analyzed in terms of their feasibility and requirement in infrastructure, human resources, land, costs, accessibility and the risks involved. In relation to coconuts, we have seen that field genebanks and *on farm* conservation represent the best conservation methods but have certain limitations in the long term (Table 2). Other options like *in vitro* techniques and cryopreservation of zygotic embryos, for example, should be pursued in the future. The advantages and disadvantages of each of the possible options (Table 2) must be weighed against each other. This kind of analysis would provide the basis for taking decisions on which conservation options to be followed for given specific objectives.

The next important step in the process would be setting up the enabling environment to allow the conservation options to be implemented. This would involve, as discussed earlier, the policy issues in terms of legislation, germplasm exchange, benefit sharing and also most importantly the sources of funding. Once these are agreed upon and put into place, a strategic action plan can be developed and implemented (steps 6 and 7). For each step, the steering committee would examine the issues and take the relevant decisions and assign responsibilities to the various relevant players.

In conclusion, a complementary conservation strategy for coconuts requires a lot of efforts and commitment from many different stakeholders, who must work together with a common objective. A proper enabling environment, including *inter alia* policy, finances, incentives and good collaborative spirit, is crucial for its success.

Table 1. Comparison of conservation options for coconuts

	In situ **on farm /Home Gardens/natural habitats**	**Botanic Gardens (Living plants in gardens/ greenhouses)**	**'Conventional' Genebanks (seed banks, field genebanks)**	**Slow growth conditions (short-term)**	**Cryopreservation - liquid N (long-term)**
Mature plants	☑ Coconuts conserved on farm widely and in home gardens and natural stands exist on small isolated islands and atolls	☑ Occurs in botanic gardens but limited scope for conserving genetic diversity	☑ Field genebank most widely used conservation method so far. National and international coconut field genebanks exist	☒Not applicable	☒Not applicable
Seeds and zygotic embryos	☒ Not feasible- seeds are recalcitrant, no natural soil seed banks	☒Not feasible	☒ Seeds are recalcitrant and too large; seed conservation not feasible	☑Field collecting protocol established for zygotic embryos; *In vitro* culture functional	☑ Cryo-preservation protocol has been established for zygotic embryos; suitable for long term conservation
Somatic embryos	☒ Not applicable	☒ Not applicable	☒ Not applicable	☒ Mass propagation problematic; Not applicable	☒ not applicable
Pollen	☒ Not applicable	☒ Not applicable	☑ Possible, for short term conservation (2-6 months)	☒Not applicable	☑ Coconut pollen can be cryopreserved and could be suitable for long term conservation
Apices	☒ Not applicable	☒ Not applicable	☒ Not applicable	☒ Not applicable	☑ cryopreservation protocol established; relatively low survival and regeneration of plants very difficult
DNA	☒ Not applicable	☒ Not applicable	☑ Storage as DNA libraries exists – value not known	☒Not applicable	☑ Long-term storage possible (LN or –80°C freezer). Use of stored DNA questionable.

Table 2. Relative advantages and disadvantages of conservation methods for coconut

Method	Advantages	Disadvantages	Research needed
Field genebank	• Easy access for characterization, evaluation and use • Simple infrastructure needs • Does not require highly skilled manpower	• Space limitation compounded by need to maintain safe isolation distance between trees, especially for the Tall types that out cross frequently • Labour intensive; High risk in mislabelling • Vulnerability to biotic and abiotic factors • Exchange of germplasm • Participation with end users difficult • Legal issues as related to land ownership	• Minimum number of palms needed to maintain representative genetic diversity • Filed plot techniques for proper characterization and evaluation • Economics of coconut field genebank maintenance
In vitro collecting and culture of zygotic embryos	• Well established protocols • Facilitates germplasm exchange	• Only short-term storage • Relatively high infrastructure needs • High maintenance cost • Less accessible to users	• Testing of optimized *in vitro* culture protocol
Cryopreservation	• Feasible for long term secure storage • Easy to maintain, low costs • Protocol for coconut embryos has been developed • Not labour intensive	• Requires skilled labour • High initial investment cost for Infrastructure	• More work required to refine cryopreservation protocol
Pollen conservation	• Large number of samples can be maintained in small space • Easy to handle • Useful for crosses • Can be cryopreserved allowing long term storage	• Not yet feasible for long term • Only conserve part of diversity • Cannot be used to conserve specific genotypes	• Refinement on cryopreservation protocol • Desiccation tolerance
On-farm	• Dynamic conservation in relation to environmental changes • Participation of local communities and stakeholders made easier • Conserve a much larger genetic diversity overall • Highly suitable for coconuts • Difficult to exchange germplasm	• Vulnerable to natural and man-directed disasters, e.g. fire, cyclones, vandalism, change in land use, deforestation etc. • Materials not easily available for utilisation • Appropriate management regimes poorly understood • Require active supervision and monitoring • Genetic diversity scattered	• Little information on status of genetic diversity across coconut stands. • Systematic documentation of farmers knowledge is needed • Several issues related to socioeconomics of coconut farming, indigenous knowledge, community participation in relation to on-farm conservation • On farm conservation methodologies need further work • Ways and means to enhance benefits for promoting conservation on farm • Piloting *in situ* methods for locating, measuring and monitoring genetic diversity

Figure 1. Framework for developing a complementary conservation strategy

Step 1 Networking of stakeholders at national, regional or international, as the case may be

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Step 2 Definition of objectives and sub-objectives

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Step 3 Analysis of the feasibility of each option for each sub-objective in terms of infrastructure needs, costs, risks involved, etc.

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Step 4 Decision on conservation options for each objective/sub-objective

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Step 5 Setting up enabling environment – policy/legal issues, funding, etc

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Step 6 Elaboration of Strategic Action Plan by stakeholder/s

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Step 7 Implementation process

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