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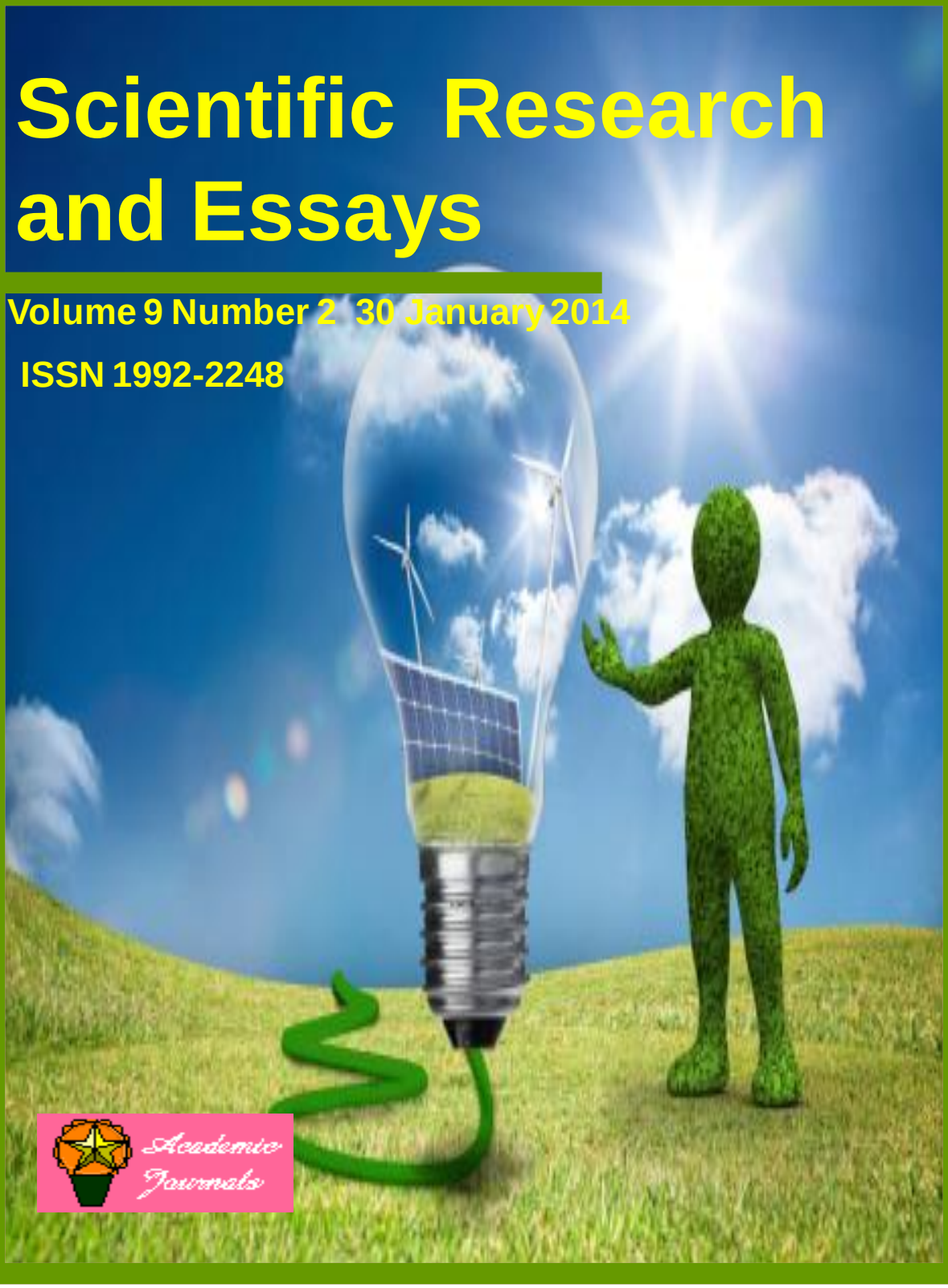

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Mundree SG, Farrant JM (2000). Some physiological and molecular insights into the mechanisms of desiccation tolerance in the resurrection plant Xerophyta viscasa Baker. In Cherry et al. (eds) Plant tolerance to abiotic stresses in Agriculture: Role of Genetic Engineering, Kluwer Academic Publishers, Netherlands, pp 201-222.

Babalola OO (2002). Interactions between Striga hermonthica (Del.) Benth. and fluorescent rhizosphere bacteria Of Zea mays, L. and Sorghum bicolor L. Moench for Striga suicidal germination In Vigna unguiculata . PhD dissertation, University of Ibadan, Ibadan, Nigeria.

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Table of Contents: Volume 9 Number 2 30 January, 2014

ARTICLES

Research Articles

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Review

Saffron (*Crocus sativus* L.) in the light of biotechnological approaches: A review

Mushtaq Ahmad*, Gul Zaffar, Mehfuza Habib, Ameeque Arshid, N. A. Dar and Z. A. Dar

Division of Genetics and Pant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar campus, Srinagar -191121, India.

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Saffron (*Crocus sativus* L.) is a sterile triploid plant belonging to the Iridaceae (Liliales, Monocots). Saffron is a spice derived from the flower and has for decades been the world's most expensive spice. Saffron is propagated by corms as the flowers are sterile and fail to produce viable seeds. A corm survives for only one season, producing up to ten "cormlets" that eventually give rise to new plants. Therefore, reproduction is human dependent; the corms must be manually dug up, broken apart and replanted. The natural propagation rate of saffron is relatively low. Biotechnological approaches have increasingly become a valuable tool assisting breeders to release new species and cultivars into the market more rapidly. Biotechnological approaches offer the capability to produce large quantities of propagating material in short time as well as the production of commercially important chemical constituents like, crocin, picrocrocin, crocetin and safranal under *in vitro* conditions. However, the protocols available so far need further refinement for their commercial utilization. Here we review the progress made in genus *Crocus*, and highlight the potential for future expansion in this field through biotechnological interventions.

Key words: *Crocus sativus* L, biotechnological approaches, sterile, corms.

INTRODUCTION

Saffron (*Crocus sativus*) which belongs to Iris family *Iridaceae* is the most expensive spice in the world and is popularly known as the “Golden Condiment”. In India it is a legendary crop of Jammu and Kashmir, produced on well drained karewa soils where ideal climatic conditions are available for good shoot growth and flower production. Plants of this family are herbs with rhizomes, corms or bulbs. The family *Iridaceae* embraces about 60 genera and 1,500 species. The genus *Crocus* includes native species from Europe, North Africa and temperate Asia, and is especially well represented in arid countries of south-eastern Europe and Western and Central Asia. Among the 85 species belonging to the genus *Crocus*, *C. sativus* L. (Saffron) is the most fascinating and intriguing species (Fernández, 2004). Dried stigmas of saffron flowers compose the most expensive spice which has been valuable since ancient times for its odoriferous, coloring, and medicinal properties (Plessner et al., 1990). The name saffron is commonly used to refer both to the spice and the plant itself. Some archaeological and historical studies indicate that domestication of saffron dates back to 2,000 to 1,500 years BC (Grilli Caiola, 2004). The origin of saffron is obscure, but the plant is believed to have originated in the eastern Mediterranean, (Winterhalter and Straubinger, 2000). Most of the *Crocus* species grow naturally in fields between shrubs and grasses or in light woodlands. The species in the genus *Crocus* have underground fleshy corms and basal, grass-probably in Asia Minor and Persia. The name ‘saffron’ is derived from Arabic *zá-faran* which means ‘be yellow’

*Corresponding author. E-mail: sahilmushtaqdar@rediffmail.com

like, dark green leaves with whitish median stripe. Their leaves appear with or after flowers. A plant may have one or several flowers. The fruit is a capsule and have numerous seeds with brownish or reddish color. Saffron is currently being cultivated more or less intensively in Iran, India, Greece, Spain, Italy, Turkey, France, Switzerland, Israel, Pakistan, Azerbaijan, China, Egypt, United Arab Emirates, Japan, Afghanistan, Iraq and recently Australia (Tasmania) (Nehvi et al*.,* 2006). Saffron has a life span of about 220 days. Rainfall in the autumn, warm summers and mild winters are the favorable climatic conditions for high yields of saffron. Water requirement of saffron is low. Garden soil (clay sand) is suitable for optimum growth of saffron. A relatively low water use, growth and development during fall and winter and a very low harvest index are some remarkable characteristics of saffron. Kashmir region in India produces between 5 to 6 t mostly dedicated to Indian's self consumption. Saffron export from India declined because sterility of saffron limits the application of conventional breeding approaches for its further improvement resulting in a low productivity (Ahmad et al., 2013). Traditional practices of saffron cultivation which ignores importance of water requirement, manual requirement, management of weeds, pests and disease and post harvest processing have been a matter of great concern particularly in Kashmir and have limited the benefits leading to a low I/O ratio. Non availability of saffron quality planting material has been another area which has affected the replantation and area expansion as the availability of quality planting material in India is less than 10%. Since more than 95,000 farm families are directly or indirectly associated with the crop in major saffron growing countries particularly in Iran and India, efforts have to be made to safeguard the interests of saffron growers by making the industry more profitable. Lack of high yielding cultivars adapted to diverse growing conditions, large area under rain fed cultivation, biotic and abiotic stresses, poor plant stand, moisture stress at terminal growth stage, inadequate seed replacement rate, poor crop management, resource poor farmers, low risk bearing capacity, inadequate input and technical support, poor infrastructure and institutional support, inefficient technology delivery system, limited policy directives and incentives and crop damage due to menace of corm rot are the important production constraints which need to be taken care off (Ahmad et al., 2013).

Saffron is a sterile triploid plant that is propagated by corms as the propagation through seed is impossible due to non setting of seeds. The natural propagation rate of most geophytes including saffron is relatively low. Besides conventional methods of propagation, biotechnological approaches such as *in vitro* cultural methods contribute importantly for the propagation of many important and economic plants. The application of contemporary biotechnological methods makes solving this problem more feasible. In view of this, a National mission on saffron was launched recently by the Government of India to ensure the revival of saffron production in Jammu and Kashmir. In 2010 the Central Government approved a plan to release Rs. 3.76 billion under this National Saffron Mission Programme for four years with the aim of improving overall saffron production, enhance its quality, build research and extension capability and develop an appropriate system for organized marketing. Premier research institutions like Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir and Central Institute of Temperate Horticulture are involved in the mission, while Development Departments of Jammu & Kashmir government are also key coordinators. Besides, many projects at these research institutions are funded under the Horticulture Technology Mission. The Indian Council of Agricultural Research is the nodal agency implementing these programs and the major thrust is on saffron corm multiplication, quality corm production, *in vitro* production of saffron/microcorms, corm rot management, saffron inter-culture, and demonstration of developed technologies to saffron growers. The Department of Biotechnology is also funding projects of individual researchers for development and refinement of saffron production and improvement technologies with a particular thrust on *in vitro* cormlet production and production of stigma-like structures. The technique, however, calls for development of convenient protocols and their standardization that will not only help in mass multiplication of elite-disease free clones but also open new vistas for application of recombinant DNA technologies for development of transgenics in this crop (Yasmin and Nehvi, 2013).

BIOTECHNOLOGICAL APPROACHES

Saffron is propagated solely by vegetative way using the annual renewal corms. Only four to five corms per mother corm are produced in one growing season through conventional methods. Hence, low multiplication rates and fungal infestation of corms are the bottlenecks for availability of sufficient quality planting material (Kiran et al., 2011). An enhancement in productivity per unit area that can lead to increase in net returns to farmers and encourage them to continue growing saffron is therefore necessary. Nevertheless, conventional breeding methodologies have not led to any improvement in saffron and alternative procedures like biotechnological, molecular biological interventions for enhancing yield and tolerance to biotic and abiotic stresses have to be explored. Tissue culture is a useful method for large scale production of disease free plants using different medium with different ratios of auxin and cytokinin (Ding et al., 1981; Chrungoo and Caiola, 1987; Ilahi et al., 1987; Plessner et al., 1990; Fakhrai and Evans, 1990;

Chen et al., 2003; Majourhay et al., 2007; Sheibani et al., 2007). However, smaller size of the tissue culture corms with least survival under actual field conditions is the limitation factor with the available tissue culture saffron protocols and further refinement for their commercial utilization is needed. Stigma-like structures were reported to be induced from almost every part of floral organs, including half ovaries (Himeno and Sano, 1987; Sano and Himeno, 1987; Loskutov et al., 1999), stigmas (Koyama et al., 1988; Sarma et al., 1990), petals (Lu et al., 1992; Jia et al., 1996), anthers (Fakhrai and Evans, 1990) and stamens (Zhao et al., 2001).

Biotechnological approaches are presently mainly used as a tool to facilitate a better understanding of the bio-chemical synthesis of saffron secondary products. Regeneration/proliferation ability of the corms was dependent on genotype, type of explants, culture initiation time and composition of the culture medium. The plants were able to form shoots or corms within 5 to 30 weeks from the start culture (GulZaffar et al., 2004). Saffron is a monocotyledon member of the large family Iridaceae. Comparatively, bulbous and cormous monocotyledons are regarded as difficult *in vitro* material. Contamination is a serious problem during micropropagation of monocots especially if underground organs, such as corms, bulbs, rhizomes and tubers are used as an explants source. The size of geophytes, physical damage and dormancy are the other problems which make tissue culture studies difficult. Schenk and Hildebrandt (1972) reported the importance of medium composition and techniques for induction and growth of monocotyledonous and dicotyledonous plants in cell culture. They found that a high level of auxin-type growth regulating substances generally favored cell cultures of monocotyledonous plants, while low levels of cytokinin were essential for most dicotyledonous cell cultures. Within the last few decades, an increasing number of bulbous and cormous monocotyledons have been successfully cultured. Tissue culture technology was greatly influenced by the demand of rapid multiplication and clonal propagation of slow-growing monocots. Several economically important monocot species constituting nutritional, medicinal or ornamental groups of plants were used for *in vitro* clonal propagation (Sutter, 1986) and production of secondary metabolites (Aslanyants et al., 1988). Organogenesis and somatic embryogenesis from differentiated tissues of bulbous and cormous monocots, such as *C. sativus* L., are also reported in the literature. Ding et al. (1979, 1981) were the first to report the successful tissue culture of *Crocus*. They successfully regenerated callus and intact plantlets from corm explants when the culture media contained indole-3-acetic acid (IAA) and 2,4-D (2,4 Dichlorophenoxyacetic acid). Later, Homes et al. (1987) observed microcorms forming on 1/8th corm explants. These regenerated shoots when cultured on a medium with 9 µM 2, 4-D (Dichlorophenoxyacetic acid) using a similar medium (Ilahi et al., 1987), produced callus on corm explants that differentiated buds. Ilahi et al. (1986) described the morphogenesis in saffron tissue culture. Corms of saffron were cultured on half strength mass medium supplemented with different combinations of growth regulators; that is, auxin and cytokinins, and coconut milk. Callus was induced in a medium containing 0.5 mg/L each of 2,4-D (2,4 Dichlorophenoxyacetic_acid) and 6-Benzylaminopurine(BAP) and 2% coconut milk. The same culture was used for differentiation of callus into buds. They reported that an increase in 2,4-D also enhanced callus formation but suppressed shoot-bud formation. These shoots were induced to root when inoculated on a medium containing 2 mg/L NAA (Naphthaleneacetic acid) for 24 h. However, further growth of these roots was slow when reinoculated on half strength MS containing 0.1 mg/L each of 2,4-D and BAP. In another set of experiments when a piece of callus, growing in similar conditions, was transferred to MS medium containing 0.5 mg/L NAA, 0.1 of either BAP or kinetin and 2% coconut milk, the nodules gave rise to roots after four weeks of culture with subsequent suppression of the shoot development. Floral organs of four spring-flowering *Crocus* species were investigated for their competence to produce callus by Choob et al. (1994). Shoot development on corm explants was promoted by cytokinins (kinetin or zeatin, 14 - 56 lM) and 2, 4-D (4.5 lM), while corm formation and growth was promoted by ethylene exposure (Plessner et al., 1990). Ovary wall explants gave the best response, with stigma and style-type structures regenerating from the explants (Choob et al., 1994). Under continuous darkness, many shoot primordial were formed. These elongated when placed in the light, and formed normal plantlets with corms (Bhagyalakshmi, 1999). Loskutov et al. (1999) studied the optimization of *in vitro* conditions for stigma-like structure production from half-ovary explants of *C. sativus*. The optimum proliferation of stigma-like structure was observed on B5 basal medium (Gamborg B5 medium) containing NAA (5.4 µM), BA (44.4 µM), MS organics, casein hydrolysate (0.05%) and L-alanine (11.2 µM). They reported that the amounts of crocin, crocetin, picrocrocin and safranal in stigma-like structure, as determined by high performance liquid chromatography analysis, were similar to those found in natural saffron. Successful stigma and ovary formation has been attained when cultured on media containing BA (4.4 to 22.2 µM) and kinetin (4.7 to 23.3 µM) (Sano and Himeno, 1987). Style and perianth explants produced stigma-like structures that proliferated forming up to 100 structures per explant (Ebrahimzadeh Karamian, 2000). Zeng et al. (2003) recorded the increased crocin production and induction frequency of stigma-like structures from floral organs of *C. sativus* by precursor feeding. MS medium supplemented with 5 mg/L kinetin and 4 mg/L NAA was used as the basal medium. Almost all of the stigma-like structures formed directly from explants, instead of from

callus. The production of non-embryogenic and embryogenic callus for the purposes of protoplast formation was investigated by Darvishi et al. (2007).

Chen et al. (2004) also examined the promotion of growth of *C. sativus* cells and the production of crocin by rare earth elements. They reported that La^{3+} and Ce^{3+}, either individually or as a mixture, promoted crocin production of *C. sativus* callus but Nd^{3+} (40 µM) had little effect and all metal ions were toxic above 100 µM. La^{3+} (60 µM) significantly promoted the growth of callus but only slightly increased crocin. Ce^{3+} (40 µM) significantly promoted crocin production but had little effect on cell growth. They showed that La^{3+} (60 µM) and Ce^{3+} (20 µM) together gave the highest dry weight biomass (20.4 g/L), crocin content (4.4 mg/g) and crocin production (90 mg/L).

Somatic embryogenesis in saffron was described also by Blázquez et al. (2004). They used MS culture medium supplemented with 0.5 mg/L BAP and 0.1 mg/L 2,4-D for induction of somatic embryogenesis. Embryogenic calli were subcultured in MS medium containing 1 mg/L BAP and 0.05 mg/L NAA for multiplication in solid medium. Temporary immersion systems (TIS) were used for this purpose. A four-fold increase in the production of embryogenic calli (fresh weight increase) was observed in TIS culture when compared to solid medium. They obtained the best result when 1 mg/L of paclobutrazol was added. They also improved the development of somatic embryos on solid medium supplemented with 0.5 mg/L jasmonic acid (JA) and obtained plant regeneration via somatic embryogenesis after eight weeks of treatment with JA in combination with sucrose. Leaf explants produced callus that regenerated somatic embryos and plantlets when cultured on 10 lM BA and 0.5 lM 2, 4-D (Raja et al., 2007). These were used for microcorm induction which was promoted by a half-strength MS medium plus 9% sucrose (Raja et al., 2007). Blázquez et al. (2004a) showed a link between type, occurrence and expression of antioxidant enzymes (superoxide dismutases and catalase) and the stage of somatic embryogenesis, suggesting these could act as markers of embryogenesis.

In vitro regenerated shoots from callus were cultured in Murashige and Skoog (MS) medium supplemented with (0.2 to 0.5 mg/L) 6-benzlaminopurine (BA), Paclobutrazol (PAC) 2 to 20 mg/L and sucrose (3 to 12%). Higher concentration of PAC (5 mg/L) along with BA (0.25 mg/L) and 9% sucrose resulted in formation of relatively large micro corms (Zaffar et al., 2012). 3C nuclear DNA of somatic embryos derived and directly formed shoots was 9.7±0.02 and 9.73±0.04 pg and 1C genome size was 4.81±0.01 x 10^9 bp and 4.80±0.01 x10^6 bp, respectively. 3C nuclear DNA and 1C genome size of mother plant was 9.77±0.02 and 4.82±0.01 x 10^9 bp, respectively indicating that genome size of tissue of raised plants remained stable (Devi et al., 2012). AP-3 gene expression was found maximum during late-preanthesis (bud) stage of flower development. Expression increases from pre-bud to bud stage and decreases from bud to flowering stage of flower development. Since AP-3 is the regulatory gene for floral development, its expression pattern determines the flowering fate in saffron (Wafi et al., 2012).

Recently, T and B-cell epitopes of Iranian *C. sativus* were mapped using bioinformatics tools and the predicted peptides were found useful for vaccine development. Expression pattern of CsLYC, CsZCD, CsBCH and CSgt-2 was studied in different flower parts and highest expression was found in stigma followed by style and petal (Mir et al., 2012). The Reverse transcriptase-PCR analysis revealed that CsZCD gene expression followed different patterns during stigma development. Highest levels of CsZCD gene expression was observed in fully developed scarlet stage of stigma. Real time PCR analysis showed that there is a sharp increase in gene expression from yellow to orange and orange to scarlet stages of stigma development. Increase in CsZCD gene expression with the development of stigma suggests its regulatory role for stigma development in saffron (Mir et al., 2012). According to (Karamian, 2004), maturated embryos could be germinated on half strength MS medium supplemented with 25 mg/L GA3. Finally, complete plantlets were obtained by transferring germinated embryos into half strength MS medium supplemented with 1 mg/L NAA and 1 mg/L BA at 20ºC under 16/8 h (light/dark) cycle. Among different types of explants, intact ovaries are more suitable for production of direct SLS than others, while the induction of indirect SLS was higher on styles than on intact and half ovaries. The best hormonal combination for induction of direct and indirect SLS was 2 mg/L kin, 8 mg/L NAA and 20 mg/LNAA, 1 mg/L BA, respectively. The HPLC comparison of natural stigma and SLS showed that all three saffron constituents are present in SLS derived, but at lower levels compared with natural ones (Ziaratina et al., 2012). Lateral and terminal meristem of plants were collected and inoculated in MS media supplemented with plus (1,2 and 4 mg/L) 2, 4-D and kinetin (0.5, 1,4 and 8 mg/L) in different combinations with 3% sucrose for callus induction after thorough surface sterilization. The first callus was induced after 35 days of inoculation from terminal meristem explants. However, lateral meristem was observed to be less responsive. The highest frequency of callus induction was achieved on MS medium supplemented with 2 mg/L 2, 4-D plus 0.5 mg/L Kin (Vahedi et al., 2012). 3C nuclear DNA of somatic embryos derived and directly formed shoots was 9.7±0.02 and 9.73±0.04 pg and 1C genome size was 4.81±0.01 x 109 bp and 4.80±0.01 x106 bp, respectively. 3C nuclear DNA and 1C genome size of mother plant was 9.77±0.02 and 4.82±0.01 x 109 bp, respectively indicating that genome size of tissue of raised plants remained stable (Devi et al., 2012). CsSERK expression is associated with induction of shoot

organogenisis and could be a potential marker for cells competent to form shoot in saffron tissue cultured *in vitro*. Also SERK gene may have a broader role in morphogenesis in cultured tissue rather than being specific to somatic embryogenesis (Vatankhan et al., 2012). Maturated embryos could be germinated on half strength MS medium containing 20 mg/L gibberellic acid (GA3). Complete plantlets with well developed root system and corm formation were obtained on transferring germinated embryos to half strength MS (Murashige and Skoog basal medium) supplemented with $5x10^{-6}$ M BA, $5x10^{-6}$ M NAA and 2% activated charcoal (Ahuja et al., 1994). MS (Murashige and Skoog basal medium Media supplemented) with 0.5 mg/L naphthalene acetic acid (NAA) and 1.5 mg/L 6-benzyl amino purine (BAP) ensured maximum bud sprouting in September with direct multiple shoot primordia initiation on 6.5 mg/L BAP in November. 6.5 mg/L BAP + 0.2 mg/L NAA resulted in maximum shoot proliferation (24); however, at higher concentration, the PGRs were detrimental in arresting the growth. Viable shoot clumps established maximum *in vitro* corms in April after sub culturing on growth retardant (CCC) at 0.25% supplemented with 9% sucrose. Sub culturing of non-flowering *in vitro* corms on growth retardant with sucrose eliminated season dependence of *in vitro* protocols in the 2nd cycle of protocol. Primary and secondary hardening before field transfer ensured 100% corm viability (Yasmin et al., 2013).

Conclusion

There is a need of expanding the area of cultivation of saffron to meet the steady increase of its demand worldwide. However, limited availability of daughter corms is one of the major hindrances for the expansion of acreage under saffron. Biotechnological approaches such as micropropagation of saffron using direct or indirect shoot induction or plantlet regeneration through somatic embryogenesis followed by microcorm production offer the capability to produce large quantities of propagating material free of disease in short duration of time. However, the protocols available so far need refinement for their commercial utilization. Alternatively, the spice saffron or its chemical constituents viz., crocin, picrocrocin, crocetin and safranal can be produced through biotechnological approaches. Among the four chemicals, production of crocin in cell cultures has been the main focus of research because of the anticancer properties of this chemical. Biotechnological approaches are the alternative to meet the worldwide demand and to preserve this “Golden Condiment”.

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Scientific Research and Essays

Full Length Research Paper

Analysing social attributes of loan default among small Indian dairy farms: A discriminant approach

Mukesh Kumar Sinha[1]*, J. P. Dhaka[2] and Biswajit Mondal[3]

[1]Directorate of Water Management, Bhubaneswar-751023, Odisha, India.
[2]National Dairy Research Institute, Karnal-132001, Haryana, India.
[3]Central Rice Research Institute, Cuttack-753006, Odisha, India.

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The study examines the socio-economic factors discriminating defaulters and non-defaulters of credit repayment. Multi-stage sampling design was adopted for selection of farm respondents. The data were collected through structured questionnaire by personal interview method. A linear discriminant function considered to examine the relative importance of different factors in discriminating between non-defaulters and defaulters. The result revealed that per capita income from crop and milk production, expenditure to total income, earning adults and off-farm income explained major share in discriminating the non-defaulters from defaulters. The mean discriminant score for the non-defaulters (Z_1) and defaulter (Z_2) were found to be 0.316 and -1.322, respectively. The critical mean discriminant score (Z) for the two groups was found to be -0.503. The high value of *Z* corresponds to non-defaulter and low value to defaulter. Later the derived classification analysis was observed that 50 out of 83 defaulters and 32 out of 37 non-defaulters were rightly classified in Z function. Thus, grouped cases classified correctly as 68.33% as factors of default. Hence, the model is found to be valid to predict whether an unknown borrower is likely to be defaulter or non-defaulter more precisely.

Key words: Discriminant function, credit, defaulter, dairy farmers.

INTRODUCTION

Animal husbandry in India play an important role in national economy and socio-economic development. Its contribution to agricultural gross domestic product (GDP) is 24.8% at current price (GOI, 2012-2013) and supports the livelihood of over 200 million rural poor (World Bank, 1999). Further, it generates continuous stream of income and employment (Nargunde, 2013; Sinha et al., 2012; Enoma, 2010) and also supports to reduce seasonality in livelihood patterns (Birthal and Ali, 2005) due to its more egalitarian distribution compared to land (Ahuja et al., 2000). Most of the milk is produced by small and marginal farmers as well as landless labourers, who owns 87.7% of the livestock (NSS, 2011). About 40 million landless poor families earns a major part of their income from milk production (World Bank, 2005), with some very limited hired labor. At the same time, farm credit and sponsored programmes is an important intervention to address the issue of rural poverty among smallholder and landless farmers (Meyer and Nagarajan, 2000). Expanding the availability of agricultural credit has been widely used as a policy to accelerate agricultural and rural development (World Bank, 2000). It is traditionally employed as a tool for providing the priority sectors with access to production inputs and enabling production to be increased. Many efforts have been made and a continuous search for sustainable interventions through appropriate credit schemes is being conducted to improve the living conditions and quality of

*Corresponding author. E-mail: mukeshwtc@gmail.com.

life of small farmers in the rural areas (World Bank, 2000). However, such efforts and interventions are often hindered by problems of repayments, which contribute to the failures of some rural credit programmes. The assumption behind credit delivery for production process is that it will generate sufficient additional income to meet the repayment obligations and have a reasonable surplus to the producers. It is in this context, the factors which influences the repayment position of borrowers assumes great significance, and identifying the potential defaulters based on social and economic parameters are of immense importance. This study is an attempt to explore the important determinants which force the small dairy farmers to default the loan repayment.

MATERIALS AND METHODS

A multi-stage purposive sampling technique was employed to select 240 households as respondents covering 120 beneficiaries and 120 non-beneficiaries of dairy loan from 3 village clusters spread over three blocks of Ranchi district of Jharkhand state. Primary data were collected by personal interview of small farmers who borrowed for dairy activities.

To examine the relative importance of different socio economic factors in discriminating between non-defaulters and defaulters, linear discriminant function analysis was used in the study. The coefficient of discriminant function measures the net effect of an individual variable, when all other variables are considered as constant. The function specified was estimated using the SPSS software.

The following functional form was used for the present analysis as shown in Equation 1:

$$Z = \sum_{n=1}^{11} I_n X_n \quad (1)$$

Where, Z = Total discriminant score for loan defaulters and non-defaulters; X_1 = Size of operational holding in acres; X_2 = Number of milch animals; X_3 = Per capita income from crop production (INR); X_4 = Per capita income from dairying (INR.); X_5 = Per capita off-farm income (INR); X_6 = Total expenditure to total income (%); X_7 = Investment in dairying (INR); X_8 = Percentage of earning adults in family; X_9 = Per capita food expenditure (INR); X_{10} = Per capita expenditure on dairy products (INR); X_{11} = Education level of family head, and I_n = (n=1,2,3,....,11) are the linear discriminant coefficients of n^{th} variable.

Two groups of equal size are required for the application of discriminant function (Bala Krishna and Iyer, 1968). In the present study, there were 37 non-defaulters and 83 defaulters; hence, a sub-sample of 37 defaulters from the total 83 defaulters was randomly taken in order to make both the groups alike for the analysis. The discriminant function was constructed by choosing the value of I_k in such a way that the ratio was equal to variation of 'Z' between groups of defaulters and non-defaulters divided by variation of `Z' within the groups of defaulters and non-defaulters, was the maximum. The calculation of the discriminant function involves the solution of the following 11 equations shown in matrix notation (Brandow and Potter, 1953):

$$SI = D \quad (2)$$

Where,

$$S = \begin{pmatrix} S_{11} & S_{12} & .... & S_{1K} \\ S_{21} & S_{22} & .... & S_{2K} \\ : & : & : & : \\ S_{k1} & S_{k2} & .... & S_{kK} \end{pmatrix} \quad I = \begin{pmatrix} I_1 \\ I_2 \\ : \\ I_k \end{pmatrix} \quad \text{and} \quad d = \begin{pmatrix} d_1 \\ d_2 \\ : \\ d_k \end{pmatrix}$$

Where, K = 11; I_k = Vector of coefficient of discriminant functions, S_kI_k = Pooled dispersion matrix, and d_k = Elements representing difference between means of two groups.

The discriminant function was tested for significance to examine whether the variables considered together were sufficiently discriminating between groups of defaulters and non-defaulters or not. The Mahalanobis D^2 test was used to measure the distance between the two groups. After transformation of the D^2 statistics, it becomes an F statistic, which was then used to see the group difference from each other.

$$D^2 = \sum_{i=1}^{11} \sum_{k=1}^{11} C_{ik} d_i d_k \quad (3)$$

$$F = \frac{N_a N_b (N_a + N_b - P - 1)}{P(N_a + N_b)(N_a + N_b - 2)} \times D^2 \quad (4)$$

Where, Cik = inverted matrix for the coefficients, D_1dk = matrix of the product of mean differences, P is the number of characteristics. The value of F is to be tested for significance with (P) and (N_a+N_b-P-1) degrees of freedom.

RESULTS AND DISCUSSION

The socio-economic characteristics of the borrowers together with means and their mean differences for the two groups of non-defaulters and defaulters of dairy loan were calculated. For this purpose, a sample of 37 defaulters and 37 non-defaulters was taken to have a valid comparison. The discriminant function for the data was estimated and presented as:

$$Z = 0.5941\ X_1 + 0.2390\ X_2 + 0.0032\ X_3 + 0.0021\ X_4 + 0.0287\ X_5 - 0.2321\ X_6 + 0.00012\ X_7 + 0.1162\ X_8 - 0.0032\ X_9 - 0.0188\ X_{10} + 0.0164\ X_{11} \quad (5)$$

The discriminant function was tested for significance to examine whether or not the characteristics considered together were sufficiently discriminating between the groups of non-defaulters and defaulters. The test of significance of discriminant function is a test of hypothesis that there are no difference in the mean values of the chosen characteristics in the two populations of non-defaulters and defaulters. D^2 and

Table 1. Factor contribution of individual characteristics to total distance measured.

S/N	Social determinants	Coefficients (l_K)	Mean difference (d_k)	Contribution of variable (l_K x d_k)	Factor contribution (%)
1	Operational holding (X_1)	0.5941	0.312	0.1853	5.78
2	Number of milch animals (X_2)	0.2390	0.270	0.0645	2.08
3	Per capita income from crop (X_3)	0.0032	167.556	0.5362	17.32
4	Per capita income from dairying (X_4)	0.0021	285.970	0.6005	19.39
5	Per capita off farm income (X_5)	0.0287	16.850	0.4836	15.62
6	Percentage expenditure to total income (X_6)	-0.2321	-2.640	0.6127	19.78
7	Capital investment in dairying (X_7)	0.0001	1581.973	0.1898	6.13
8	Percentage of earning adults (X_8)	0.1162	2.990	0.3474	11.22
9	Per capita food expenditure (X_9)	0.0032	-30.108	-0.0963	-3.11
10	Per capita expenditure on dairy items (X_{10})	-0.0188	-8.241	0.1549	5.00
11	Educational status (X_{11})	0.0164	-6.092	0.0179	0.005
	Total			3.0965	100.00

variance ratio were worked out and found to be 5.0654 and 5.0931, respectively. Since the tabulated value of F statistics ($F_{11,\ 62}$) at 5% level is 2.49, the discriminant function was found to be significant. This indicates that the eleven characteristics considered together are useful in classifying the borrowers into the groups of non-defaulters and defaulters. In order to examine the relative importance of characteristics based on their power to discriminate between the two borrower groups, the percentage contribution of each character to the total distance measured were calculated and the results are exhibited in Table 1. The results revealed that the characteristics like per capita income from crop production (17.32%), per capita income from dairying (19.39%), per capita off-farm income (15.62%), percentage expenditure to total income (19.79%) and the percentage of earning adults (11.29%) were the major characteristics, which led to classify the borrowers into two groups of defaulters and non-defaulters.

The students 't' test was calculated for testing the mean difference between the groups for each variable and they exhibited significant t' values for the above identified variable at 5% level. The variables like per capita income from crop production (X_3), per capita income from dairying (X_4), per capita off-farm income (X_5), percentage expenditure to total income (X_6), and percentage of earning adults to total family (X_8) were observed to be significant. Hence, these variables were judged as the major attributes which discriminate the borrowers into non-defaulters and defaulters.

The discriminant function was again re-run by taking only those five significant variables in the equation to see whether these characteristics alone could discriminate the defaulters and non-defaulters significantly or not and it was observed from this analysis that these characteristics were very useful for measuring distance in the discriminating power. The new discriminating function taking only the significant factors was estimated as:

$$Z = 0.023\ X_3 + 0.0011\ X_4 + 0.0038\ X_5 - 0.0636\ X_6 + 0.0212\ X_8 \quad (6)$$

Again, the discriminant function was tested to examine whether these characteristics considered together are significantly discriminating between the groups of defaulters and non-defaulters. The D^2 and variance ratio were worked out to be 4.6241 and 5.3154, respectively. Since the tabular value of $F_{(5,62)}$ at 5% level is 4.43, the discriminant function is significant. This implies that the five characteristics considered together were useful in classifying the borrowers into the groups of non-defaulters and defaulters. Thus, the difference in the groups was mostly oriented towards per capita income, percentage expenditure to total income and percentage earning adult to the family. The discriminating variables obtained are quite contrary to the variable chosen by George et al. (1984), Lekshmi et al. (1998) and Gandhimathi (2012), while the results obtained were in conformity with findings of Bandyopadhyay (2006), Nawai and Shariff (2010).

Further, the relative importance of the characteristics to discriminate between the two groups of borrowers, the percentage contribution of each variable to the total distance measured were examined and the results are exhibited in Table 2. The magnitude of the coefficient of the function is an indicator of the relative importance of individual variable. The coefficient in the Z equation suggest that higher per capita income from crop production, higher income from dairying, percentage expenditure to total income, off-farm income sources and more earning adults in the family contributed high value of Z, explained major share in discriminating the non-defaulters from defaulters followed by percentage earning adults and off-farm income. The weights associated with these characteristics to the total distance measured were obtained as 38.72, 31.62, 16.87, 6.43 and 6.36, respectively.

The discriminant function was later used to predict

Table 2. Relative importance of significant characteristics for defaulter and non-defaulter.

S/N	Socio-economic variables	Coefficients (l_K)	Mean difference (d_k)	Contribution of variable (l_K x d_k)	Factor contribution (%)
1	Per capita income from crop (X_3)	0.0023	167.556	0.3853	38.72
2	Per capita income from dairying (X_4)	0.0011	285.970	0.3146	31.62
3	Per capita off farm income (X_5)	0.0038	16.850	0.0640	6.43
4	Percentage expenditure to income (X_6)	-0.0636	-2.640	0.1679	16.87
5	Percentage of earning adults (X_8)	0.0212	2.990	0.0633	6.36
	Total			0.9951	100.00

Table 3. Classification analysis results (confusion matrix) of the borrower groups.

Actual group	Number of cases	Predicted group membership			
		Defaulters	%	Non-defaulters	%
Defaulters	83	50	60.24	33	39.76
Non-defaulters	37	5	13.52	32	86.48

whether a borrower is likely to be a non-defaulters or defaulters. The mean discriminant score Z_1 for the non-defaulters and defaulters Z_2, that is, sum of the products of the coefficient and corresponding mean value of significant characteristics, were found to be 0.316 and -1.322, respectively. The critical mean discriminant score (Z) for the two groups was found to be -0.503. This implies that, if the discriminant score for a respondent on the basis of significant variable is found to be more than -0.503, the respondent can be predicted to be non-defaulters, otherwise he is likely to be a defaulters. The high value of Z corresponds to non-defaulter and low value to defaulter. It will be interesting to see that what proportion of respondents considered in the present study is rightly classified by the function. With this criterion, the whole sample of 120 respondents (borrowers) was classified into defaulters and non-defaulters. Then, it was compared with the actual discriminant classification. This classification is called as derived classification analysis.

The percentage of cases classified correctly is an indicator of the productive power of fitted discriminant function, while evaluating this measure, it is important to consider the observed mis-classification rate to that by chance. It was seen from the Table 3 that 50 out of 83 defaulters and 32 out of 37 non-defaulters were rightly classified in the Z function. The number of respondents wrongly classified was 38 out of 120 respondents of defaulters and non-defaulters. Thus, grouped cases classified correctly as 68.33%. Therefore, the model is found to be valid to predict whether an unknown borrower is likely to be defaulter or non-defaulter, more precisely.

CONCLUSION AND RECOMMENDATION

Farm credit and supported programme for dairy development is a policy intervention to improve livelihoods of small and marginal farmers as well as landless people. The study investigated the localized social factors that should be used by the lender in risk rating their farm customer. The study suggests that lower income from the crop production, dairy and off -farm activities coupled with high expenditure proportionate to income and less number of earning adults in the family leaves lesser surplus with the farmer that makes them to default. The derived classification analysis cross verified the predicted variables and found that the group classified correctly by 68.33% as factors of default. Thus, the model is considered as valid in predicting the defaulters based on localized factors precisely. The results will address the concerns of the lenders in advance to assess the credit risks of their capital and risk adjustment for serving larger group of small holding/farmers. Therefore, lending agencies must take note of important social as well as other locally important variables and social characteristics before granting the loan to small holder to reduce the incidence of loan delinquencies and defaults.

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Scientific Research and Essays

Full Length Research Paper

Evolution characteristics and driving forces of wetland changes in the Poyang Lake eco-economic zone of China

Hualin Xie[1]*, Qu Liu[1] and Guiying Liu[2]

[1]Institute of Poyang Lake Eco-Economics, Jiangxi University of Finance and Economics, Nanchang 330013, China.
[2]School of Economics and Management, Jiangxi Agriculture University, Nanchang 330045, China.

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Wetland ecosystem is known as the "kidney" of earth and the "gene pool" of species. It has the functions of regulating climate, flood storage and degradation of pollution. In this paper, based on GIS technology and landscape ecology, wetland changes and its driving forces in the Poyang Lake Eco-economic Zone of China are analyzed. The analysis of landscape pattern demonstrates that there is an increase in the degree of fragmentation of wetlands in the study area. At the same time, the overall aggregation degree of the lake is in the rise. The increased Perimeter-area fractal dimension indicates that the shape of wetland becomes more and more rules. The main driving forces of wetland changes in the Poyang Lake Eco-economic Zone include natural factors and human activities. This study also indicates the main natural factor is the changes of precipitation, though for now, there is increase of average temperature. At the moment, the rapid population growth, regional economic development and other human activities are also the key driving forces of wetland landscape changes in the Poyang Lake Eco-economic Zone.

Key words: Wetland change, landscape pattern, climate change, ecosystem management, Poyang Lake.

INTRODUCTION

Land use/land cover change (LUCC) in the field of global environmental change research has been gaining increasing degree of attention because of its role in the social and ecological environment (Vitousek, 1997; Li, 1996). As one of the important land types, wetlands are also increasingly becoming of widespread concern by many scholars (Huang et al., 2012; Nagabhatla et al., 2012; Scott and Metts, 2012). Wetland ecosystem, which is known as the "kidney" of earth and the "gene pool" of species, has become one of the most productive ecosystems on the planet (Whigham, 1999; Cserhalmi et al., 2011). Wetland is not only a valuable natural resource for human survival, it is also one of the most important environments (Kingsford, 2011). It not only directly provides the raw material for production and human life, it performs some environmental functions such as regulating climate, flood storage, control of pollution as well as degradation etc (Traill et al., 2010). Analysis of the driving force of various wetland changes is one of priorities and focuses of wetlands LUCC research (Akin et al., 2012). The dynamic monitoring and evaluation of wetlands and its driving force have become the hotspot of current wetland changes research (Behera et al., 2012; Jiang et al., 2012; Kayastha et al., 2012; Landmann et al., 2013). At present, a large number of studies show that the driving forces of wetland changes concerns climate change, vegetation and human disturbance (Guardiola-Albert and Jackson, 2011; Lopez-Merino et al.,

*Corresponding author. E-mail: xiehl_2000@163.com. Tel: +86-791-8381-0957. Fax: +86-791-8381-0892.

2011; Miller et al., 2012; Zhen et al. 2011). Urbanization is an irresistible trend of the development of human society in the 21st century. Most arable lands have been converted to construction land by urban expansion in China. This made a large number of ecological land including wetlands, which plays a pivotal role in ecological service function, exploited to arable land for meeting the objective of arable land protection. The newly formed wetlands in western China were caused primarily by climate warming over that region whereas the newly created artificial wetlands were caused by economic developments (Gong et al., 2010). Therefore, it is meaningful to explore the mechanisms of wetlands evolution.

Poyang Lake Region of China is recognized as one of the fundamental ecological function districts by the World Wide Fund for Nature (WWF). It plays a vital role in the provision of fresh water resources, the maintenance of the regional water balance, the homogenization of the flood, the regulation of regional climate and the conservation of biological resources (Deng et al., 2011; Yan et al., 2013). In recent years, some activities including reclamation of land from the lake and agricultural development have made wetland of Poyang Lake region change dramatically, bringing about increasing obvious ecological problems (Chen and Chen, 2012; Feng et al., 2012; Shankman and Liang, 2003). In view of the high service value of wetland and the vast eco-environment effect of LUCC, it is necessary to study process and mechanism of wetland in the Poyang Lake Eco-economic Zone (Huang et al., 2012). The Mountain-River-Lake Program (MRL) was implemented since 25 years ago in the Poyang Lake basin, southern China. It consists of series of forest restoration projects that aim to address severe soil and water losses, and improve farmer's livelihoods (Huang et al., 2012). Therefore, the study of changes in wetlands in the Poyang Lake Region of China becomes increasingly urgent. As we all know, the study of wetland landscape pattern can better understand the ecological processes. Exploring the change of natural wetland landscape pattern over time and revealing its driving forces are an urgent need to study the issue for the Poyang Lake (Huang et al., 2012; Yan et al., 2013).

The main purposes of this study are: 1) how to study the characteristics of wetland changes based on the theories and methods of landscape ecology; 2) to explore the evolution pattern of different kinds of wetlands; 3) and to find the driving forces of wetland changes in the Poyang Lake Eco-economic Zone for Sustainable Watershed Management.

MATERIALS AND METHODS

Study area

The study area (28°30′N to 30°06′N, 114°29′E to 117°25′E) is located in Jiangxi Province, a southern region of China, with a surface of approximately 51,200 km^2 (Figure 1). The area belongs to the subtropical humid climate zone, with an annual average temperature of 16 to 18°C and an annual average rainfall of 1,600 mm. Annual average sunshine is about 1,473.3 to 2,077.5 h. Annual sunshine total radiation is about 97 to 114.5 Kcal/cm^2. Soils are predominantly red soil, yellow soil and paddy soil. Poyang Lake is the largest freshwater lake in China and is one of the six wetlands with rich biodiversity in the World. Taking Poyang Lake as the core and relying on the Poyang Lake city circle, the Poyang Lake Eco-economic Region is the significant economic zone for protecting the ecology and developing economics. The study area includes 38 counties and has a population of 20.06 million and GDP of 3,948.17 billion Yuan (RMB) in 2008. One of goals of the study area is to build an international demonstration zone for the harmonious ecological and economic development.

Data

Land use data of 1990, 2000 and 2005 employed in this study came from the 1:100,000 national land use database of the Data Center for Resources and Environmental Sciences, Chinese Academy of Sciences (RESDC). Wetland types in this study are divided into three classes and 11 subclasses (Table 1). Based on the ArcGIS9.3 software, land use data resampled at the spatial resolution of 100 m × 100 m. Climatic data came from the China Meteorological Data Sharing Service System. Social-economical data at the county level in this study derived from the Jiangxi province statistics yearbook from 1985 to 2006.

Land dynamic degree

Land dynamic degree is used to measure the number of changes in the situation for some time within certain land use types. The formula is given as:

$$K = \frac{U_b - U_a}{U_a} \times \frac{1}{T} \times 100\% \quad (1)$$

Where K is the dynamic degree of a certain land types within the study period; U_a and U_b respectively represent the area of land use types in the beginning and at the end of the study; T is the length of the study period. When T is set for the year, the value of K is the average annual rate of the area change of a certain land type.

Transfer matrix of land use change

On the basis of the transfer matrix of land use types, transition probability matrix of land use types is established to describe the changes in the intensity of land use types. The formula is given as:

$$D_{ij} = \sum_{ij}^{n} [\frac{dS_{i-j}}{S_i}] \times 100\% \quad (2)$$

Where D_{ij} is the transition probability of land use type *i* converted into land use type *j* in the study period; S_i is the total area land use type *i* of the beginning of the study; dS_{i-j} is the sum of the areas of land use type *i* converted into the land use type *j* in the study period; n is the number of land use types changed in the study area.

Conversion contribution ratios

The transfer matrix method describes the evolution of different land

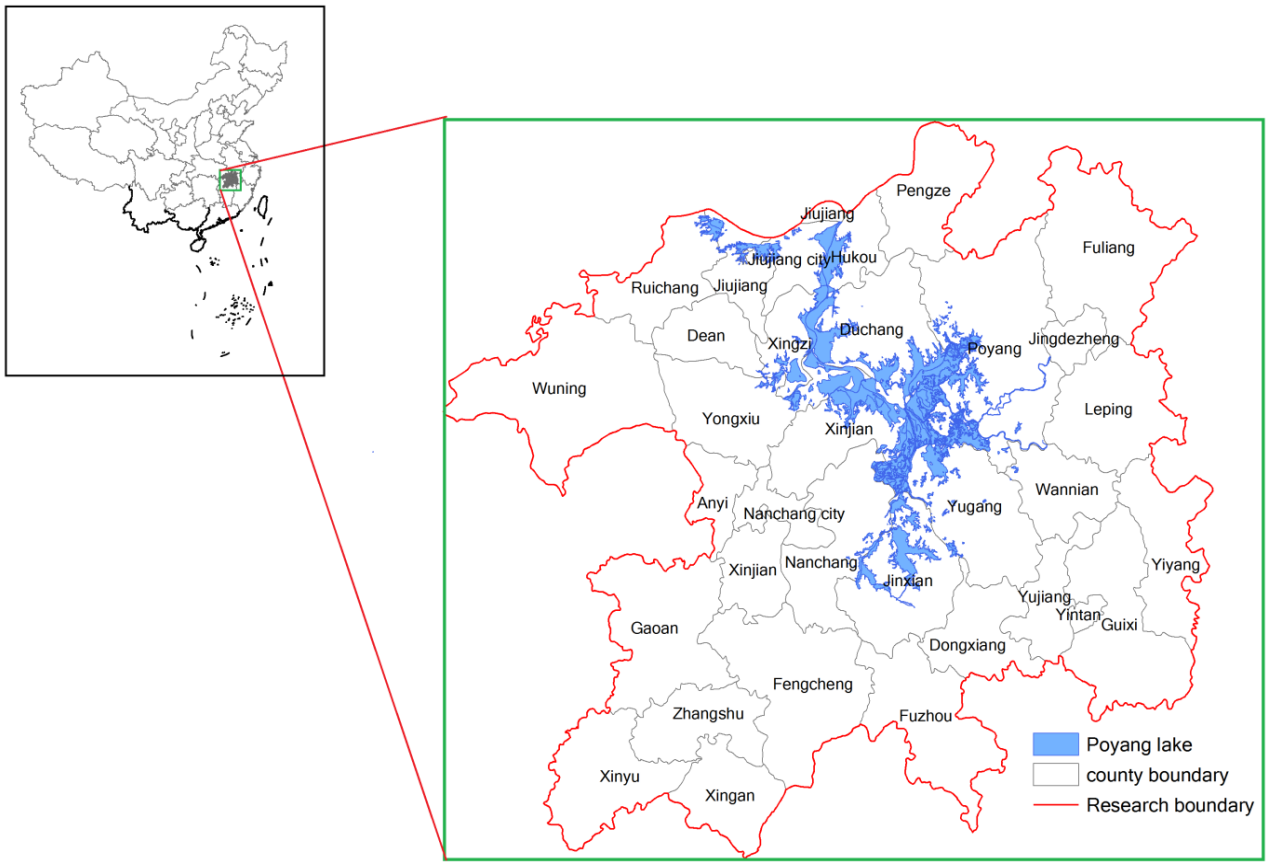

Figure 1. Location map of Poyang Lake Eco-economic Zone in China.

Table 1. Wetland classes of the study area.

Wetland class	Wetland subclass
Natural wetland	Lake
	River
	Lakeshore
	Swamp
Artificial wetland	Paddy field
	Reservoir and pond
Non-wetland	Forest
	Grass
	Dry field
	Constructed land
	Other non-wetland

use types. In order to fully reflect the status and role of information of different land types in the land use pattern, the method of conversion contribution ratios of transfer to/from the land use types is conducted in this study. It can comparatively analyze spatial pattern and quantity characteristics of the transfer-in and transfer-out of the various types of land use. The formula of conversion contribution ratios of transfer-in of land use types is given as:

$$L_{ji} = \sum_{j=1}^{n} S_{ji} / S_t \quad (3)$$

Where L_{ji} means the proportion of the area of other kinds of land use types except i converted into land use type i accounting for the total area transferred; S_{ji} refers to the transfer area of the land use type j converted to land use type i; S_t is the total area of land use type transferred; n is the number of land use types. L_{ji} can be used to compare the area differences of increment allocated for the various kinds of land in the transfer-in process of land dynamic change.

The formula of conversion contribution ratios of transfer-out of land use types is given as:

$$L_{0j} = \sum_{j=1}^{n} S_{ij} / S_t \quad (4)$$

Where L_{0j} means the proportion of the area of land use type i converted into other kinds of land use types accounting for the total area transferred; S_{ij} refers to the transfer area of the land use type i converted to land use type j; S_t is the total area of land use type transferred; n is the number of land use types. L_{0j} can be used to compare the area differences of decrement allocated for the various kinds of land in the transfer-out process of land dynamic change.

Landscape pattern analysis

Landscape ecology can provide new theories and methods for a comprehensive solution to the resource and environmental problems and to carry out a detailed ecological environment construction. In this study, we selected seven landscape indices to reflect the characteristics of the wetlands changes at landscape and class level.

Number of patches of a particular patch type is a basic measure of the extent of subdivision or fragmentation of the patch type. The formula of Number of Patches (NP) is given as:

$$NP = n_i \quad (5)$$

Where *NP* represents the number of patches; n_i is the number of patches in the landscape of patch type (class) *i*.

Patch density has the same basic utility as the number of patches as an index, except that it expresses the number of patches on a per unit area basis that facilitates comparisons among landscapes of varying size. The formula of Patch Density (PD) is given as:

$$PD = \frac{n_i}{A} \quad (6)$$

Where *PD* represents the patch density; n_i *is the* number of patches in the landscape of patch type (class) *i*; A is the total landscape area.

Largest Patch Index at the class level quantifies the percentage of total landscape area comprised by the largest patch. As such, it is a basic measure of dominance. The formula of Largest Patch Index (LPI) is given as:

$$LPI = \frac{Max(a_1, \cdots, a_n)}{A} \quad (7)$$

Where *LPI* represents the Largest Patch Index; a_i is the area of patch (class); A is the total landscape area.

Perimeter-area fractal dimension is appealing because it reflects shape complexity across a range of spatial scales (patch sizes). A fractal dimension greater than 1 for a 2-dimensional landscape mosaic indicates a departure from a Euclidean geometry (that is, an increase in patch shape complexity). The formula of Perimeter-area fractal dimension (PAFRAC) is given as:

$$\ln(P/4) = k\ln(A) + c, PAFRAC = 2k \quad (8)$$

Where *PAFRAC* represents the perimeter-area fractal dimension; *A* is the area of patch (class); *P* is the perimeter of patch (class).

Landscape division is based on the cumulative patch area distribution and is interpreted as the probability that two randomly chosen pixels in the landscape are not situated in the same patch. The formula of Landscape Division Index is given as:

$$DIVISION = \left[1 - \sum_{i=1}^{M}\sum_{j=1}^{N}\left(\frac{a_{ij}}{A}\right)^2\right] \quad (9)$$

Where *DIVISION* represents the Landscape Division Index x; a_i is the area of patch (class); A is the total landscape area.

Shannon's Diversity Index is a popular measure of diversity in community ecology, applied here to landscapes. The formula of Shannon's Diversity Index (SHDI) is given as:

$$SHDI = -\sum_{i=1}^{m}[P\ln_i(P_i)] \quad (10)$$

Where *SHDI* represents the Shannon's Diversity Index; p_i is the proportion of the landscape occupied by patch type (class) *i*.

Aggregation index is calculated from an adjacency matrix, which shows the frequency with which different pairs of patch types (including like adjacencies between the same patch type appearing side-by-side on the map. The formula of Aggregation Index (AI) is given as:

$$AI = [\frac{g_{ii}}{\max \rightarrow g_{ii}}](100) \quad (11)$$

Where *AI* represents the Aggregation Index; g_{ii} is the number of like adjacencies (joins) between pixels of patch type (class) *i* based on the single-count method.

RESULTS AND DISCUSSION

Overall analysis of wetland dynamic changes

The areas and its changes of different kinds of wetland during the period 1990 to 2005 in the Poyang Lake Eco-economic Zone are listed in Table 2. From Table 2, we can see that there was an increasing trend of natural wetland and the artificial wetland in the study area showed a decreasing trend from 1990 to 2005. For natural wetland, lake increased from 200,523 hm^2 in 1990 to 284,300 hm^2 in 2005, an increase of 41.78%. As can be seen from the Figure 2, there is a rapid growth of the construction land. Figure 2 also shows that the increase in the lake is more obvious from 1990 to 2005 due to the conversion of a large area of the lakeshore.

Figure 3 shows the dynamic degree of different wetland types during the period 1990 to 2005 and the period 2000 to 2005 in the Poyang Lake Eco-economic Zone. As can be seen from the Figure 3, the value of dynamic degree of the lake is largest, 9.22 during the period 2000 to 2005. The value of dynamic degree of paddy field is smallest, -0.05 during 1990 to 2005. The value of dynamic degree of most wetland types is negative except lake.

The transfer matrix of different types of wetlands from 1990 to 2005 is listed in the Table 3. Many lakes, rivers, lakeshores, reservoir and ponds transformed into paddy fields, the area respectively 2955, 189 and 3072 hm^2 (Table 3). This means that many natural wetlands were replaced by the artificial wetlands. As can be seen from the Table 3, 34% of the paddy fields were pushed back the natural wetlands, and 25% of the paddy fields were occupied by the constructed land during the period 1990 to 2005 in the Poyang Lake Eco-economic Zone. At the same time, there is a greater conversion between the different types of wetlands. The main types mainly transferred to the lakes are the beaches, marshes and rivers.

The conversion contribution ratio of different kinds of

Table 2. Wetland change during the period 1990-2005 in the Poyang Lake Eco-economic Zone.

Wetland type	Area/hm^2			2005-1990	
	1990	2000	2005	Value change/hm^2	Rate of change/%
Natural wetland	**508836**	**517904**	**520518**	**11682**	**2.30**
Lake	200523	194575	284300	83777	41.78
River	83208	83230	75164	-8044	-9.67
Lakeshore	133724	149184	99822	-33902	-25.35
Swamp	91381	90915	61232	-30149	-32.99
Artificial wetland	**1725821**	**1712303**	**1690627**	**-35194**	**-2.04**
Paddy field	1602788	1599564	1591100	-11688	-0.73
Reservoir and pond	123033	112739	99527	-23506	-19.11
Non-wetland	**3042230**	**3046680**	**3064455**	**22225**	**0.73**
Forest	2204498	2205681	2204023	-475	-0.02
Grass	218270	216548	212752	-5518	-2.53
Dry field	478998	474293	470504	-8494	-1.77
Constructed land	139852	149526	176544	36692	26.24
Other non-wetland	612	632	632	20	3.27

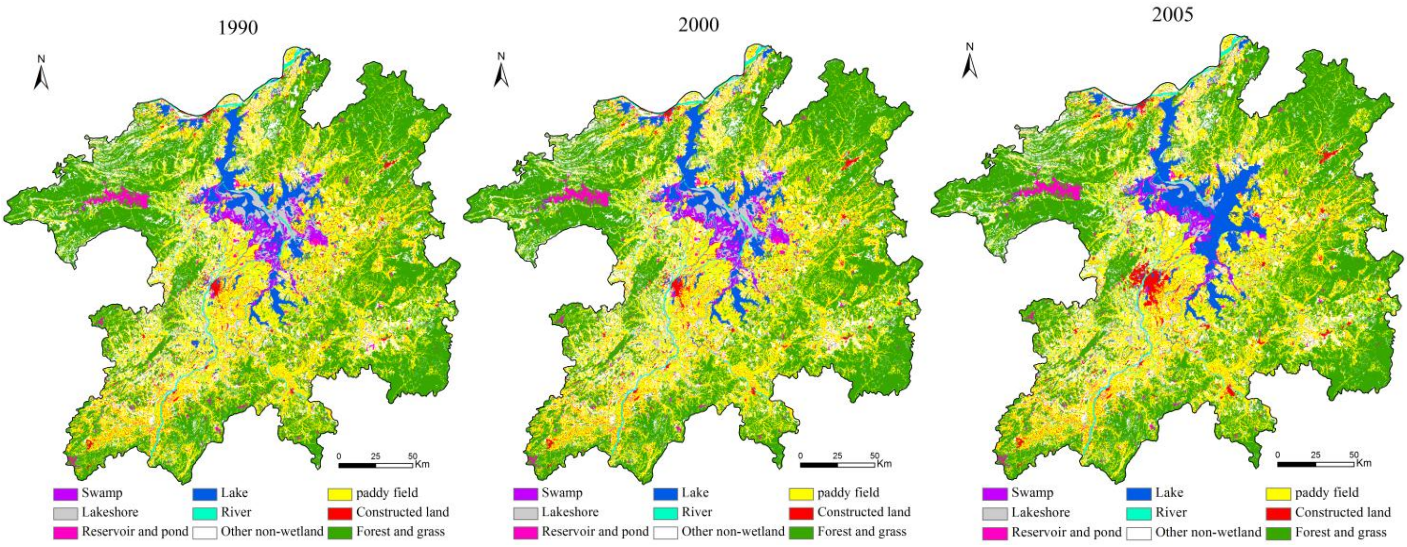

Figure 2. Spatial distribution of wetlands of Poyang Lake Eco-economic Zone in 1990, 2000 and 2005.

wetlands from 1990 to 2005 is listed in the Table 4. Compared with the contribution ratios of other transfer-in landscape components, the contribution ratios of transferring into the lake are the largest, 62.18% during the period 2000 to 2005 (Table 4). While the contribution ratio of transfer-in is less than transfer-out during the period 1990 to 2000, in general, the contribution ratio of transferring into the lake is greater than transferring out of the lake. This is because a large number of lakes have been reclaimed into the paddy fields during the period 1990 to 2000.

The concern is that the contribution rate of transferring into or out the landscape components directly or indirectly is controlled by the change of the lake in the Poyang Lake Eco-economic Zone. During the period 1990 to 2000, the conversion contribution ratio of transferring into the lakeshore is the maximum, 42.11% because large areas of ponds and lake converted into lakeshore. During the period 1990 to 2005, the conversion contribution ratio of transferring into the lake is the maximum, 51.22% due to

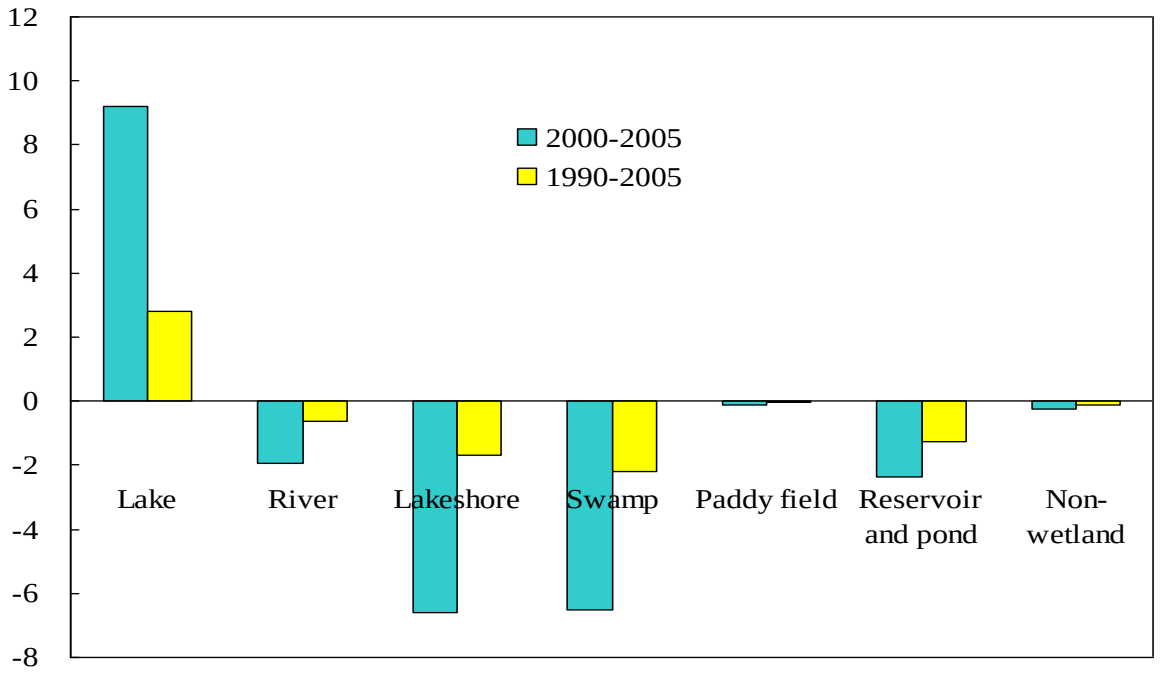

Figure 3. Dynamic degree of different wetland types during the period 1990-2005 and the period 2000-2005 in the Poyang Lake Eco-economic Zone.

Table 3. Transfer matrix of different types of wetlands from 1990 to 2005 (hm^2).

1990-2005	Lake	River	Lakeshore	Swamp	Paddy field	Reservoir and pond	Forest	Grass	Dry field	Constructed land	Other non-wetland
Lake	-	4	4834	61	2955	278	14	399	200	520	0
River	8197	-	285	2	189	35	4	0	71	63	0
Lakeshore	48248	123	-	21	3072	2152	367	1057	272	627	0
Swamp	31694	37	50	-	125	125			277	77	0
Paddy field	1920	597	3660	1304	-	3893	1544	13	3129	19926	5
Reservoir and pond	2036	1	12063	306	14059	-	370	358	1320	1095	0
Forest	136	23	120	12	1579	581	-	5106	2558	5958	63
Grass	399	0	402	119	984	215	9501	-	343	652	0
Dry field	398	21	615	219	1152	779	4330	161	-	9076	0
Constructed land	16	3	15	194	876	52	34	3	108	-	0
Other non-wetland	0	0	0	0	0	0	48	0	0	0	564

Table 4. Conversion contribution ratio of different kinds of wetlands from 1990 to 2005 (%).

Period	Conversion type	Lake	River	Lakeshore	Swamp	Paddy field	Reservoir and pond	Non wetland
1990-2000	Transfer-in	3.77	0.44	42.11	0.23	17.93	20.10	15.41
	Transfer-out	17.08	0.39	7.53	1.27	25.14	43.13	5.46
2000-2005	Transfer-in	62.18	0.41	3.72	1.46	13.56	1.81	16.85
	Transfer-out	1.44	5.87	37.13	21.55	18.83	10.75	4.43
1990-2005	Transfer-in	51.22	0.45	12.14	0.51	13.76	4.46	17.46
	Transfer-out	5.10	4.87	30.80	17.83	19.10	17.40	4.91

Table 5. Change of landscape indices of whole wetland from 1990 to 2005.

Year	NP	PD	LPI	PAFRAC	DIVISION	SHDI	AI
1990	15974	0.7148	9.7702	1.5132	0.9743	1.0361	82.0775
2000	16249	0.7286	9.7818	1.5151	0.9743	1.0361	81.9827
2005	15988	0.7231	9.9693	1.5216	0.9689	0.9942	82.4600

the large number of the lakeshore and swamp converted into lake. During the period 1990 to 2000, the conversion contribution ratio of transferring out the paddy field is the maximum, 25.14% due to the large areas of paddy field converted into constructed land. Because of the large areas of lakeshore converted into the lake, the conversion contribution ratio of transferring out the lakeshore is the maximum, 37.13% during the period 2000 to 2500 and 30.8% during the period 1990 to 2000. This is mainly because of the increase in rainfall during this period.

Pattern change of wetland landscape

The change of landscape indices of whole wetland in the Poyang Lake Eco-economic Zone from 1990 to 2005 is listed in Table 5. As can be seen from Table 5, the patch number of wetland landscape increased from 15974 in 1990 to 15988 in 2005, which showed an increasing trend in the Poyang Lake Eco-economic Zone. At the same time, Landscape Division Index (DIVISION) showed a downward trend from 1990 to 2005. It means that the separation degree of wetland increased. According to the changes of Number of Patches (NP) and Landscape Division Index (DIVISION), we can infer that there is an increase in the degree of fragmentation of wetlands in the study area.

Average fractal dimension index means the self-similar degree of the patch, to some extent, and it can reflect the impact degree of human activities on the patch. The Perimeter-area fractal dimension increases from 1.5132 in 1990 to 1.5216 in 2005 (Table 5). The increase of average fractal dimension means that the shape similarity of wetland landscape patch increases and the shape become more and more rules. This is mainly because the large number of marsh wetlands reclamation becomes more regular paddy fields.

From Table 5, we can see that Shannon's Diversity Index (SHDI) decreased from 1.0361 in 1990 to 0.9942 in 2005. Shannon's Diversity Index (SHDI) can reflect the landscape heterogeneity and is extremely sensitive to the non-equilibrium distribution of each patch type in the landscape. It emphasizes the contribution of rare patch types of information. The decrease of Shannon's Diversity Index shows that wetland type in the regional landscape is more monotonous, and the contribution of information of rare patch types reduced.

The result of landscape indices of different kinds of wetlands in 1990 and 2005 is listed in Table 6. In respect of the lake wetland, as can be seen from Table 6, the number patches of the lake are in decline, and the largest patch index shows an upward trend from 1990 to 2005.

The largest patch index (LPI) can reflect the effect degree of the maximum patch on the entire landscape. The increase in the largest patch index of lake indicated that the degree of Poyang Lake landscape controlling the whole wetland showed an enhanced trend. Meanwhile, Aggregation Index (AI) of lake increased from 92.7145 in 1990 to 94.6958 in 2005. It shows that the overall aggregation degree of wetland is in the rise.

As for the swap wetland, the largest patch index shows a downward trend from 1990 to 2005. Meanwhile, the Perimeter-area fractal dimension decreased from 1.4568 in 1990 to 1.4296 in 2005 (Table 6). It indicates that the large patch of wetlands is fragmented and becomes increasingly irregular. The increase in the number of patches and reduction in patch density of swamp also supports this conclusion.

As for the reservoir and pond, the patch number and largest patch index shows a downward trend from 1990 to 2005. Meanwhile, the Aggregation Index (AI) decreased from 72.37 in 1990 to 71.84 in 2005. The Perimeter-area fractal dimension increased from 1.4297 in 1990 to 1.4415 in 2005 (Table 6). This means that pond wetland is fragmented and becomes increasingly regular by the human disturbance.

As can be seen from Table 6, for all wetlands, the patch number and patch density of paddy field is largest. This means that the paddy field is the largest wetland type interfered by human. Aggregation degree of the lake is highest, which means that the connectivity of the lake is the best. Overall, the patch number of artificial wetlands is greater than natural wetlands. Simultaneously, the patch density and separation degree of artificial wetlands is greater than natural wetlands. This is mainly a result of manual interference. Table 6 shows that the connectivity of natural wetlands is higher than artificial wetlands.

Driving forces of wetland change

The main driving factors of wetland changes in Poyang Lake Eco-economic Zone include natural factors and human activities. Natural factors usually include climate, geology, geomorphology, hydrology, vegetation, soil, and so on. Human activities are mainly reflected in the

Table 6. Landscape indices of different kinds of wetlands in 1990 and 2005.

Type	Year	NP	PD	LPI	PAFRAC	DIVISION	SHDI	AI
Lake	1990	482	0.0091	0.7929	1.3749	98.7594	0.9999	92.7145
	2005	447	0.0085	3.1949	1.3987	99.5089	0.9989	94.6958
River	1990	691	0.0131	0.6528	1.6611	98.7521	1.0000	75.0698
	2005	636	0.0121	0.5283	1.6534	98.5275	1.0000	75.2963
Lakeshore	1990	1292	0.0245	0.2730	1.4515	96.0796	1.0000	83.6639
	2005	1926	0.0365	0.0829	1.4614	92.2784	1.0000	77.2215
Swamp	1990	213	0.0040	0.4486	1.4568	98.5830	1.0000	89.2230
	2005	181	0.0034	0.3696	1.4296	98.4530	1.0000	89.3645
Paddy field	1990	8053	0.1526	4.1375	1.5515	99.4854	0.9956	81.3159
	2005	8178	0.1550	4.1784	1.5504	99.4812	0.9957	81.34
Reservoir and pond	1990	5243	0.0994	0.4455	1.4297	94.7593	1.0000	72.3708
	2005	4620	0.0876	0.4785	1.4415	95.5238	1.0000	71.8425

demographic, economic, and policy aspects. Natural factors often have a role in the landscape at the larger spatial and temporal scales. In other words, environmental backgrounds control the main changes of wetland. Meanwhile, factors of human activities are the main driving force of the dynamic changes of the wetland at a shorter time scale. Wetland is a special type in the watershed landscape. Water is the fulcrum needed to maintain its ecological structure, function and spatial characteristics of landscape and is the main carrier of material, energy and information flow within the wetlands and other land type. Water environment is the motivating factors directly promoting the formation and evolution of wetland.

Atmospheric rainfall becomes the main replenishment water for the wetland. Thus precipitation directly impacts on the regional wetland area. Figure 4 shows the change of annual mean precipitation in Poyang Lake Region from 1981 to 2010. According to Table 2, lake decreased from 200,523 hm^2 in 1990 to 194,575 hm^2 in 2000, then increased 284,300 hm^2 in 2005. At the same time, annual precipitation decreased from 1543 mm in 1990 to 1455 mm in 2000, then increased in 2005 to 1472 mm (Figure 4). There is a strong correlation between the average annual rainfall and the area of Poyang Lake. This is mainly because a lot of lakeshore land turned into lakes when annual precipitation is abundant.

In addition to the effects of precipitation, temperature changes are also critical factors that affect the wetland landscape changes. Temperature not only affects the vegetation growth status and biomass, it also affects the process of evaporation, intensity of surface and surface evaporation. Figure 5 shows the change of annual mean temperature in the Poyang Lake Region from 1981 to 2010.

As can be seen from Figure 5, in general, the annual average temperature in the Poyang Lake Eco-economic Zone shows a rising trend. From Figure 3, we can see that the average annual rainfall of Poyang Lake Ecological Economic Zone shows an overall decreasing trend. Reduced rainfall will reduce the water supply of upstream river runoff on wetlands, reduce soil moisture, exacerbates the drought level, eventually leading to the degradation of the swamp. The rise in temperature will increase the surface evaporation, affecting the wetland area. Meanwhile, some rivers shrinking dry and the many bubble marsh shrink or disappear due to reduction of water. Therefore, the changes of annual rainfall and temperature become the main driving forces of natural wetlands change.

With the rapid economic development, over-exploitation of wetland resources is the main reason for the loss of a large area of wetlands in the Poyang Lake Eco-economic Zone. This is mainly because the economic development and population growth will inevitably lead to the rapid increase of the construction land and other non-wetlands, which occupied more natural wetlands, especially the beaches.

According to the analysis related statistics, during the period 1990 to 2005, the non-agricultural population of the Poyang Lake Eco-economic Zone grows rapidly from 3,591,700 in 1990 to 5,678, 500 in 2005, an average annual growth of 139,100. Accordingly, the proportion of the non-agricultural population increased from 21.3% in 1990 to 28.3% in 2005. The proportion of the non-agricultural population growth reflects a trend of

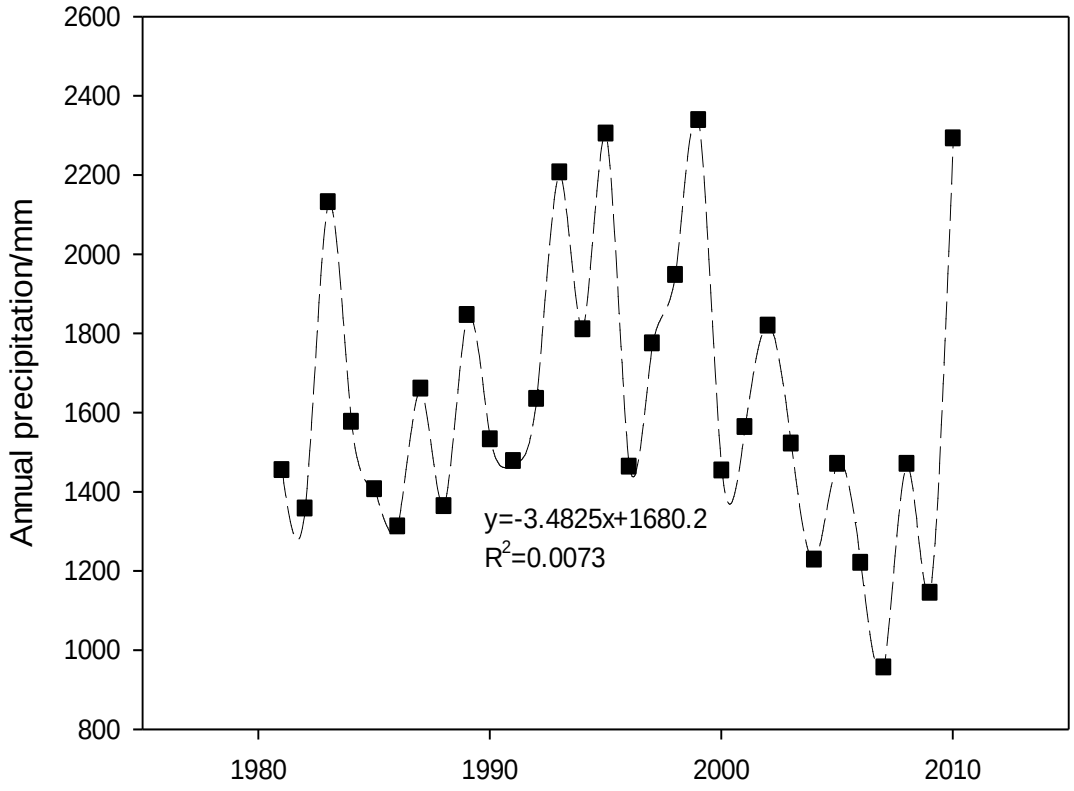

Figure 4. Annual mean precipitation of Poyang Lake Region from 1981 to 2010.

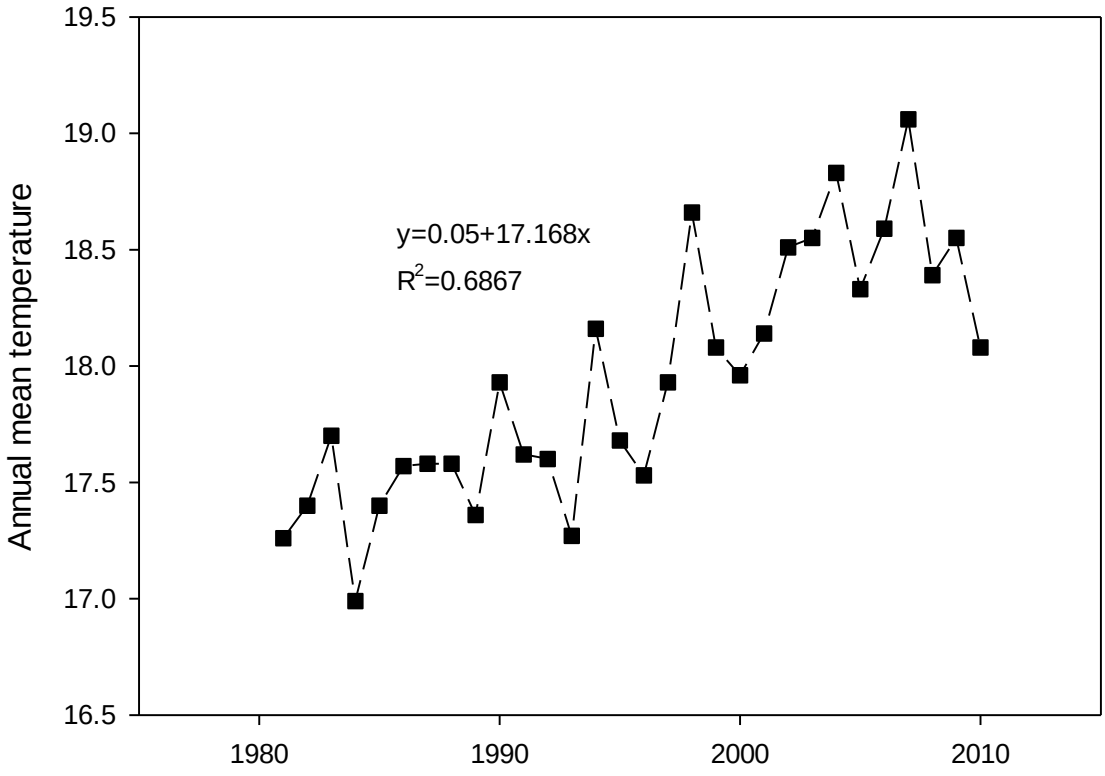

Figure 5. Annual mean temperature of Poyang Lake Region from 1981 to 2010.

urbanization and industrialization. Therefore, industrialization and urbanization are also extremely crucial driving forces of wetland changes in the Poyang Lake Eco-economic Zone. Industrialization and urbanization in the study area has made the land use of non-farm through the population concentration, industry concentration and the geographical spread of occupied land. As the weak link in the land use structure, wetland has become the most obvious type of land use change in the non-agricultural conversion process. In the past 15 years, a large number of ponds and paddy fields occupied construction land in the study area, which can be seen from Table 3. Therefore, a large number of natural wetlands have gradually reclaimed the artificial wetlands or artificial landscape in the interference of human activities for economic benefits. This is mainly in the agricultural development activities such as paddy field development and urban construction such as transportation and urban settlements.

Some policies have a profound impact on the wetlands change of Poyang Lake Eco-economic Zone, especially in playing a key role in the process of protecting their reasonable evolution. Since the late 1980s, the study area is in the implementation of the Mountain River Lake Development Program, which is a watershed integrated management program for the sustainable development of Poyang Lake watershed. The object of the project is to achieve the harmonious development of the economy, society and environment. It has formed a governance guideline "Governing lakes must be preceeded by first regulating the rivers, regulating the rivers must be preceeded by first managing mountains, managing the mountains must lead to poverty reduction". Implementation of the project has protected the water quality of Poyang Lake and reduced the degradation of wetlands.

Other policies like “cultivated land balance” has been negative on the wetland changes of Poyang Lake Eco-economic Zone. In the context of increasing demand for construction land, swamps and ponds are facing threat of agricultural development because of meeting the requirements of “cultivated land balance”. Forest restoration projects implementation and farmers’ livelihoods improvement are the important measures to protect wetlands in the Poyang Lake Eco-economic Zone. How to use quantified methods, such as correlation analysis, factor analysis, and so on, to explore the driving forces of wetland changes is important for future research.

Conclusions

There is an increasing trend of natural and artificial wetlands in the study area which shows a decreasing trend from 1990 to 2005. The value of dynamic degree of the lake wetland is largest, 9.22 during the period 2000 to 2005. The main types mainly transferred to the lakes are the beaches, marshes and rivers during the period1990 to 2005.

The contribution ratio of transferring into the lake is the largest, 62.18% during the period 2000 to 2005. Overall, the contribution ratio of transferring into the lake is greater than transferring out the lake from 1990 to 2005. During the period 1990 to 2005, the conversion contribution ratio of transferring into the lake is the maximum, 51.22% due to the large number of the lake and swamp converted into lake. 25% of the paddy fields were occupied by the constructed land during the period 1990 to 2005 in the Poyang Lake Eco-economic Zone.

The analysis of landscape pattern indicates that there is an increase in the degree of fragmentation of wetlands in the study area. The Perimeter-area fractal dimension increased from 1.5132 in 1990 to 1.5216 in 2005, which indicates that the shape of wetland becomes more and more rules. The Aggregation Index (AI) of lake increases from 92.7145 in 1990 to 94.6958 in 2005, which means that the overall aggregation degree of the lake is in the rise. The aggregation degree of the lake is highest, which means that the connectivity of the lake is the best.

The change of temperature and precipitation of the study area has a significant impact on wetland changes. The rapid population growth, regional economic development and other human activities are also the key driving forces of wetland landscape changes in the Poyang Lake Eco-economic Zone.

The above conclusions in this study provide the basis for the sustainable management and decision-making of the Poyang Lake Watershed.

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Scientific Research and Essays

Full Length Research Paper

Development of UV digestion unit for natural rubber latex preparation before the determination of phosphorus residue with artificial neural network-digital image-based colorimetry

Prinya Masawat[1]*, Benyanan Panwong[2] and Yuthapong Udnan[3]

[1]Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, Thailand.
[2]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand.
[3]Research Center for Academic Excellence in Petroleum, Petrochemicals and Advanced Materials, Naresuan University, Phitsanulok, Thailand.

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Digital image–based colorimetry (DIC) coupled with artificial neural network (ANN) was studied for the determination of phosphorus in natural rubber (NR) latex. The method is based on the RGB (Red, Green, and Blue) value of an image of phosphorus standard solution reacted with molybdenum blue solution. The picture of the complex solution was captured by using a complementary metal oxide semiconductor (CMOS) camera. Because of a colloidal property of NR latex, phosphorus residue could not be directly determined by this technique. Therefore, a UV digestion unit was designed and fabricated for the digestion of NR latex before color developing and processing with DIC-ANN. The digestion time needed for complete digestion was 50 min. The digestion capability of the UV digester to process 10 samples simultaneously led to an acceptable samples analysis frequency of 12 h^{-1}. Quantitative measurement of phosphorus was made in the range 0.1 to 1.0 mg L^{-1}. The proposed method was successfully applied to the determination of total phosphorus in NR latex and it was found to be simple, rapid, accurate, precise and low cost method.

Key words: Digital image-based colorimetry, artificial neural network, UV digestion, natural rubber latex, phosphorus.

INTRODUCTION

Natural rubber (NR) latexis a major agricultural product of Thailand due to the hot climate being conducive to the growth of the rubber tree. Thai rubber latex industries such as rubber manufacturing plants, gloves, condoms, tires, etc. require a high quality of NR latex. One parameter in the pricing of NR latex is the residual amount of magnesium. Magnesium is a chemical that affects the stability of NR latex. Therefore, it is necessary to precipitate magnesium before manufacturing process. Diammonium hydrogen phosphate (DAHP) or diammonium phosphate (DAP) is used for the precipitating process. Then magnesium sediment (magnesium ammonium phosphate) is removed in the form of sludge by centrifugation. The excessive use of DAHP or DAP causes phosphate residues in NR latex. The phosphate residues will react with some chemicals in production process effecting the formation of the products (Karunanayake and Perera, 2006; Patthanakul et al.,

*Corresponding author. E-mail: prinyam@nu.ac.th. Tel: +66 (55)963439. Fax: +66 (55)963401.

2009). Therefore, the detection of phosphate residues is very important. However, phosphate in NR latex consists of many types such as phospholipids, free orthophosphate, sugar phosphate and phosphate from precipitation of magnesium (Loadman, 1998). Thus, the total phosphate is determined in the form of total phosphorus in this research. Recently, a new technique called digital image–based colorimetry coupled with artificial neural network (DIC-ANN) was developed in our laboratory for protein assay in NR latex and medical latex gloves which could be used instead of spectrophotometry (Bang-iam et al., 2013). Unfortunately, phosphorus residues in NR latex cannot be directly determined by this technique because the NR latex has a colloidal characteristic which obstructs the penetration of light beams. In addition, the procedure for color developing by the molybdenum blue method involves certain acidic chemicals (American Public Health Association, 1992). When the NR latex is exposed to acid, it becomes agglomerated. Thus, digestion of NR latex before the determination of phosphorus in NR latex is a crucial step. The digestion process is a procedure for destroying the rubber constituents until achieving a clear solution which is ready for the color developing process. It normally uses energy such as heat (UV light, microwave, thermoreactor) or chemical reagents such as acids or a combination of the two methods (Matusiewicz, 2003; VelpScientifica, n.d.). In previous studies, the phosphorus content in environmental and pharmaceutical samples was determined using ammonium persulphate or potassium persulphate as the oxidizing agentor used in conjunction with acid such as perchloric acid or sulfuric acid and with UV light providing the energy (Benson et al., 1996; Tzanavaras and Themelis, 2003; Tue-Ngeun et al., 2005a, b). For NR latex sample, a previous study used the Kjeldahl technique to obtain the clear and appropriate solution (Loadman, 1998). Concentrated sulfuric acid and nitric acid were used in the digestion procedure together with high pressure and temperature. There are no findings on the sample preparation of NR latex using UV-assisted digestion before the detection of phosphorus with molybdenum blue method. Consequently, the purpose of this research was to develop a simple UV-assisted digestion unit for the preparation of NR latex before the determination of total phosphorus residues by molybdenum blue spectrophotometric method and digital image–based colorimetry coupled with artificial neural network (DIC-ANN). In the paper, a description of the fabrication is given and the optimal conditions are explored. Initial results are presented based on real NR latex samples.

EXPERIMENTAL

Chemicals and reagents

All chemicals were of analytical grade and all solutions were prepared by using deionized (DI) water (Prima Reverse Osmosis, Maxima water purification system, Elga Ltd., England). A stock solution (1,000 mg L^{-1}) of phosphorus was prepared by dissolving 0.4390 g of KH_2PO_4 (Rankem, India) in DI water and diluting to 100 ml in a volumetric flask. The standard phosphorus solution was stored at 4°C. This solution is stable for 1 month. Solution of 30 g L^{-1} ammonium peroxodisulphate ($(NH_4)_2S_2O_8$, LobaChemie, India) was prepared by dissolving 3.0 g in DI water and adjusting to a volume of 100.0 ml. Solution of potassium antimonyl tartrate ($K(SbO)C_4H_4O_6.1/2H_2O$, Riedel-de Haen, Germany) was prepared by dissolving 0.27 g in DI water and adjusting to a volume of 100.0 ml. Solution of ammonium molybdate ($(NH_4)6Mo_7O_{24}.4H_2O$, Ajax Finechem, Australia) was prepared by dissolving 4.0 g in DI water and adjusting to a volume of 100.0 ml. Solution of ascorbic acid ($C_6H_8O_6$, Rankem, India) was prepared by dissolving 1.76 g in DI water and adjusting to a volume of 100.0 ml. The ascorbic acid solution is stable for 1 week at 4°C. Solution of 5 N sulfuric acid (H_2SO_4, Carlo ERBA, Italy) was prepared by diluting 35.0 ml to a volume of 250.0 ml with DI water. Molybdenum blue solution was prepared by mixing 5 N sulfuric acid solution, potassium antimonyl tartrate solution, ammonium molybdate solution and ascorbic acid solution in the ratio of 10:1:3:6, respectively (American Public Health Association, 1992). Stock solution (1,000 mg L^{-1}) each interfering ion was prepared by dissolving an appropriate amount of the suitable salt.

5.0 g each of the NR latex samples (high ammonia latex, 60% of dry rubber content, Thai Rubber Latex Corporation, Public Company Limited, Thailand) were stirred to evaporate ammonia for 20 min. The sampleswere stored in polyethylene tube and were freshly prepared for daily use.

Apparatus

A doublebeam UV-Vis Spectrophotometer (Lambda 20 Perkin Elmer, USA) was used for the optimization of the proposed digestion method and also results comparison with the DIC-ANN measurement. A digital image-based colorimeter (under Petty Patent since 2012, Thailand) coupled with an ANN-program (under copyright since 2011, Thailand) utilized in this work was fabricated and gave satisfactory results for protein assay in NR latex and medical latex gloves (Bang-iam et al., 2013). The data for calibration set and real samples sets were obtained by taking images in the DIC light box. The ANN program which was trained with the back-propagation of errors learning algorithm, was used to extract the red (R), green (G), and blue (B) values from the images. The ANN has three input nodes (one for each of the R, G, B channels), two hidden layers with 11 nodes (because the output is the concentration of standard phosphorus from 0-1.0 mg L^{-1}), and one output node (phosphorus concentration). In contrast to UV-Vis spectrophotometry, the construction of calibration curve is not required when using DIC-ANN. After ANN training (random learning for 3,000 iterations) of the standard phosphorus images, the program could directly predict the concentration of the phosphorus residue in the unknown NR latex samples.

Laboratory-made UV digestion unit

A schematic diagram of the system assembled for UV digestion unit (under Petty Patent since 2013, Thailand) acquisition is illustrated in Figure 1. A digestion unit was made from a 35 × 24 × 21 cm (width × height × depth) black cardboard adapted from a first aid box (ESM Medical, Thailand) to maintain the temperature and UV light in the closed system. The UV lamp (300 W, Osram, Slovakia) was secured on the wall of the box and in front of the ten screw cap test tubes (16 mm) which was located in the stainless steel rack. The temperature can go up to 160± 5°C after warming up for 10 min. The distance between the UV lamp and the screw cap test

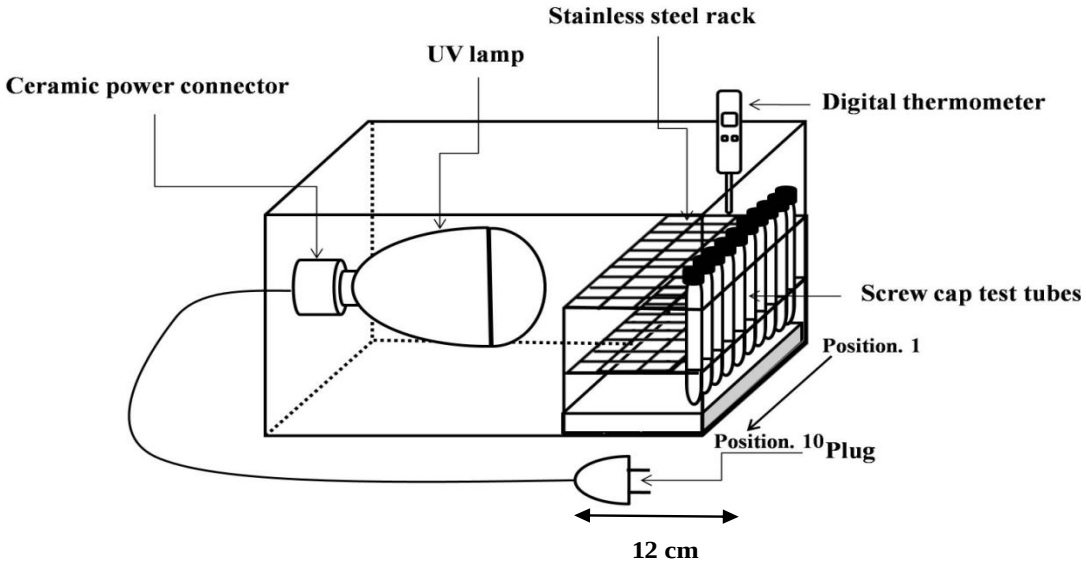

Figure 1. Schematic diagram of a laboratory-made UV digestion unit.

tubes at the back row of the rack is 12 cm. A thermometer was placed at the top of the box in order to monitor the digestion temperature throughout the experiment.

The digestion tube position is likely to have great effect on the reproducibility of the results. The fabricated UV digestion unit used a stainless steel test tube rack as the sample holder. The distance between the UV lamp and the first row of the rack is 3 cm. Ten digestion tubes were placed in the end row of the rack (12 cm from the UV lamp) because the first four rows at the front are too near the UV lamp causing vigorous boiling of solution. Thus, in this experiment, digestion tube positioning was studied in ten positions at back row of the rack. The results indicated that all positions provide no difference in normalized absorbance. It was confirmed by the standard deviation (SD) and the relative standard deviation (RSD) which was 0.23 absorbance g^{-1} and 2.14%, respectively. Therefore, the digestion tube positioning at all positions could be used for NR latex digestion.

RESULTS AND DISCUSSION

In this work, the UV digestion unit was constructed and applied to NR latex digestion coupled with the oxidizing agent. Five parameters including type of oxidizing agent, effect of oxidizing agent on phosphorus molybdenum blue complex spectra, concentration of oxidizing agent, digestion time and reaction time for color development were studied and optimized. Normalized absorbance was used for the study of these effects because of the viscosity of NR latex. It is difficult to accurately pipette the NR latex thus the sample weighing was carried out and normalized absorbance which means absorbance divided by weight of NR latex was utilized instead of absorbance.

The effect of types of oxidizing agent

Types of oxidizing agents were studied in order to select the optimum oxidizing agent for the decomposition of biological matrices in NR latex. The use of oxidizing agents such as hydrogen peroxide, nitric acid, sulfuric acid, perchloric acid, ammonium peroxodisulphate and potassium peroxodisulphate were reported for various samples in the literature reviews (Benson et al., 1996; Golimowski and Golimowska, 1996; Zhang et al., 1996; Lyengar et al., 1998; Tzanavaras and Themelis, 2003; Tue-Ngeun et al., 2005a, b). Non oxidizing acid were chosen in this study because the NR latex is coagulated during treatment with acid. Thus, the strong oxidizing agents such as ammonium peroxodisulphate and potassium peroxodisulphate were considered instead. The phosphorus standard solutions in the concentration range of 0 to 4 mg L^{-1} were spiked with NR latex samples followed by adding oxidizing agent before digestion. The slope when using ammonium peroxodisulphate and potassium peroxodisulphateas oxidizing agent for the NR latex digestion shows similar results. Thus, it can be concluded that the sensitivity is unaffected by the choice of these oxidizing agents. However, the solubility of ammonium peroxodisulphate is higher than potassium peroxodisulphate. Moreover, ammonium peroxodisulphate is cheaper than potassium peroxodisulphate. Therefore, ammonium peroxodisulphate was chosen as oxidizing agent for the next study.

The effect of oxidizing agent on phosphorus molybdenum blue complex spectra

Under the optimum conditions, the effect of the ammonium peroxodisulphate used as oxidizing agent on the absorbance of phosphorus molybdenum blue

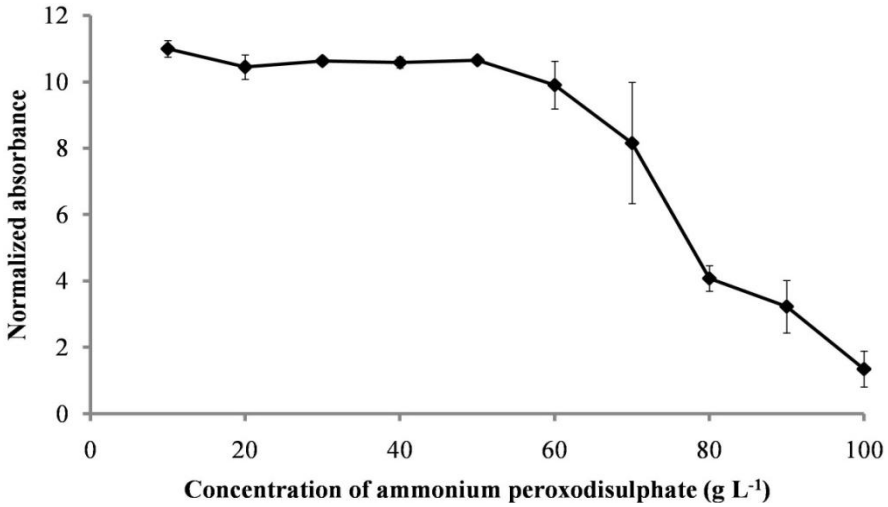

Figure 2. Effect of concentration of ammonium peroxodisulphate used as oxidizing agent coupled with UV-assisted digestion on normalized absorbance.

complex in digested NR latex sample was studied by scanning the wavelength in the range of 400 to 900 nm. The spectrum of the complex compound indicated the maximum wavelength at 710 and 880 nm that is similar to the spectra of phosphorus standard solutions. It can be concluded that the oxidizing agent has no effect on the complex compound of phosphorus in NR latex with molybdenum blue solution. Thus, the maximum wavelength at 880 nm was chosen for the next experiment.

The effect of concentration of oxidizing agent

The effect of concentration of the oxidizing agent for NR latex digestion by UV digestion unit was studied in the range of 10 to 100 g L^{-1}. From Figure 2, the signal is constant up to 50 g L^{-1} of ammonium peroxodisulphate solution. Then the signal decreased with increasing the concentration of the oxidizing agent from 60 to 100 g L^{-1}. However, the solutions obtained using 10 to 20 g L^{-1} of the oxidizing agent are not clear and they have a lot of pieces of the coagulated rubber particles owing to incomplete decomposition reaction. Therefore, a filtration step was carried out in the sample preparation. Moreover, the phosphorus complex compound might be adsorbed on the filter paper. The best result was obtained when using 30 g L^{-1} of the oxidizing agent providing clear solution and low reagent consumption. Therefore, concentration of the oxidizing agent at 30 g L^{-1} was selected for the next study.

The effect of digestion time

The UV digestion unit (after a 10 min warm-up) operates at a constant temperature of 160 ± 5°C. However, the digestion time has the effect on the rate of decomposition reaction. The digestion time was studied in the range of 10 to 60 min. Figure 3 shows that the normalized absorbance increased with increasing digestion time, nevertheless a plateau was reached after 20 min. Moreover, the resultant solutions at the range of 20 to 30 min are not clear and the standard deviation at 40 min is higher than that at 50 min. Therefore, the digestion of NR latex for 50 min was selected in the next experiment.

The effect of reaction time for color development

After the NR latex digestion, the reaction time of the colorimetric reaction between phosphorus and molybdenum blue reagent was studied in the range of 5 to 60 min. The results of 0.3 mg L^{-1} of phosphorus standard solution, NR latex sample with and without added phosphorus standard (0.02 mg g^{-1}) were obtained as illustrated in Figure 4. It was found that, all colorimetric reactions are stable after 10 min. Therefore, the standing time for 10 min before phosphorus determination was chosen for the next experiment.

Analytical performance for the determination of total phosphorus in NR latex

The linear range

The linear range of phosphorus standard solution was studied in the range of 0.1 to 1.0 mg L^{-1} for the determination of total phosphorus in NR latex sample. Under the optimum conditions, the results of the calibration solutions set without digestion procedure and

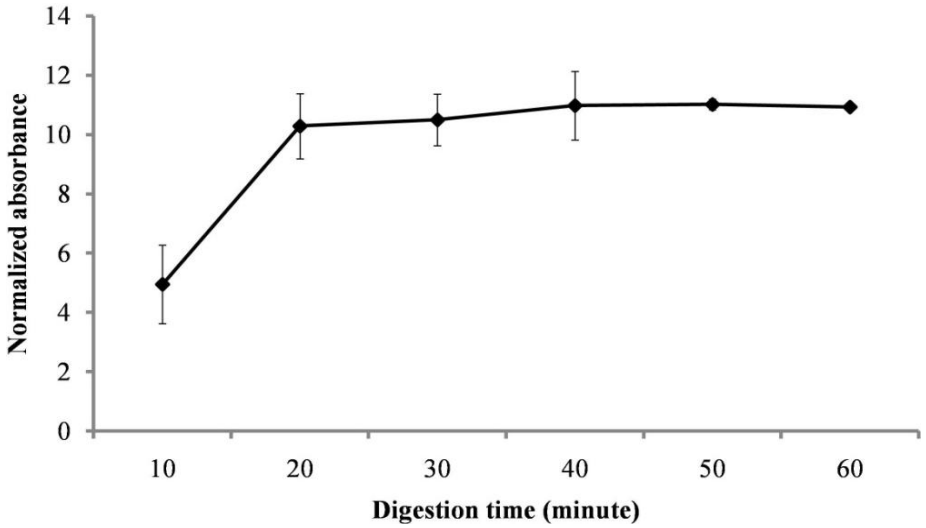

Figure 3. Effect of digestion time of NR latex sample on normalized absorbance by using UV-assisted digestion couple with 30 g L^{-1} ammonium peroxodisulphate.

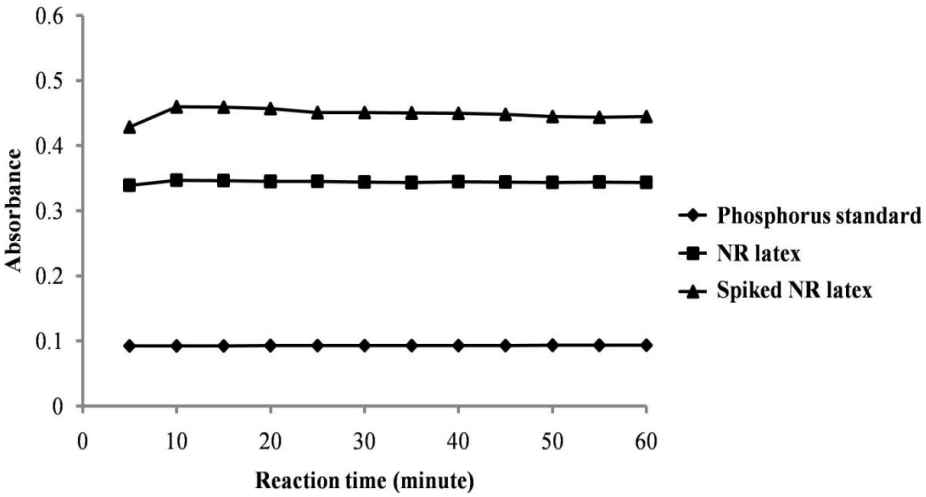

Figure 4. Effect of reaction time for color developing of phosphorus standard solution (0.3 mg L^{-1}), phosphorus in NR latex sample after digestion using UV digestion unit and spiked (0.02 mg g^{-1} of phosphorus standard) NR latex sample after digestion using UV digestion unit with molybdenum blue solution.

with digestion procedure in which 5 ml of 30 g L^{-1} oxidizing agent was added before coupled with UV digestion unit. It was found that the digestion step has little effect on calibration curves and the slope values of these curves are not significantly different. It can be concluded that the calibration method with digestion and without digestion are equally sensitive. Nevertheless, the construction of calibration solutions without the digestion step reduces preparation time, energy and reagent. Therefore, the calibration curve construction without the digestion step was selected for total phosphorus estimation in NR latex sample.

The recoveries for the determination of total phosphorus in NR latex sample

NR latex samples were digested using UV digestion unit and analyzed for recoveries of the total phosphorus by UV-Vis spectrophotometry. NR latex containing added phosphorus standard solutions were analyzed and represented in Table 1. The recoveries of total phosphorus using the UV digestion unit are in the range of 84.7 to 103%. These recoveries indicated that the proposed digestion techniques can be used for the determination of total phosphorus in NR latex.

Table 1. Recoveries of UV digestion unit for the analysis of total phosphorus in NR latex samples.

Samples	Added (mg g^{-1})	Found (mg g^{-1}, n=3)	% Recovery[a]
1	0	0.176	-
	0.01	0.186 ± 0.0006	103 ± 5.77
2	0	0.171	-
	0.02	0.187 ± 0.0005	84.7 ± 2.70
3	0	0.164	
	0.03	0.190 ± 0.0010	86.7 ± 3.33
4	0	0.168	-
	0.04	0.206 ± 0.0010	95.0 ± 2.50
5	0	0.163	-
	0.05	0.207 ± 0.0015	87.3 ± 3.00

[a]Mean value ± standard deviation (n = 3).

Table 2. Effect of interfering ions on recovery of total phosphorus.

Interfering ions	Tolerance limit concentration (mg kg^{-1})	%Recovery [a]
AsO_4^{3-}	0.001	94.1 ± 7.29
S^{2-}	1.0	97.7 ± 6.42
NO_2^-	10.0	93.8 ± 1.61
Cr^{6+}	1.0	90.3 ± 4.58
SiO_3^{2-}	1.0	85.8 ± 1.73

[a] Mean value ± standard deviation (n =3)

The effect of interfering ions on recoveries of total phosphorus determination

The effect of potential interferences upon the molybdenum blue reaction was studied at 0.02 mg g^{-1} phosphorus ions in NR latex samples. Quantitative recoveries of the analyte were shown in Table 2. The presence of arsenate (AsO_4^{3-}), sulfide (S^{2-}), nitrite (NO_2^-), hexavalent chromium (Cr^{6+}) and silicate (SiO_3^{2-}) can interfere with the process of determining the phosphorus content (American Public Health Association, 1992) when interfering ions concentration are higher than the concentration as presented in Table 2.

Validation of the method

Under the chosen conditions described above, by using UV-Vis spectrophotometry, the calibration graphs were linear over the range 0.1 to 1.0 mg L^{-1}.The value of LOD ($3SD_{blank}$ / slope, n=15) was 3.14×10^{-4} mg L^{-1} and LOQ ($10SD_{blank}$ / slope, n=15) was 1.1×10^{-3} mg L^{-1}. The relative standard deviations (%RSD) of intraday and interday analyses for recoveries of phosphorus using the laboratory-made UV digestion unit were 2.48 and 5.98%, respectively (n=7).

The validation and estimation performance of an ANN prediction model were normally determined by mean square error (MSE) or root mean square error (RMSE) or mean absolute deviation (MAD) or mean absolute percentage error (MAPE) (Elmolla et al., 2010; Roush et al., 2006; Salle et al., 2003; Twomey and Smith, 1996; Yetilmezsoy and Demirel, 2008). Therefore, the accuracy and precision of the ANN written program used in this work were validated by MSE and the relative standard error (%RSE).It was found that the average MSE from phosphorus standard solutions testing at 0.1, 0.3, 0.5, 0.7 and 0.9 mg L^{-1} was 0.0020. The low MSE indicated that the accuracy of the DIC-ANN is relatively high. Thus, the DIC-ANN could be used for the determination of total phosphorus in NR latex. The %RSE is the standard error expressed as a fraction of the estimate value and is usually displayed as a percentage. The high %RSE is subject to high sampling error. The %RSE was 0.05 when reading the RGB values for 3,000 times. This indicated that the proposed method gave high precision. Therefore, the numbers of iteration for 3,000 times were selected for the RGB values reading in the ANN written

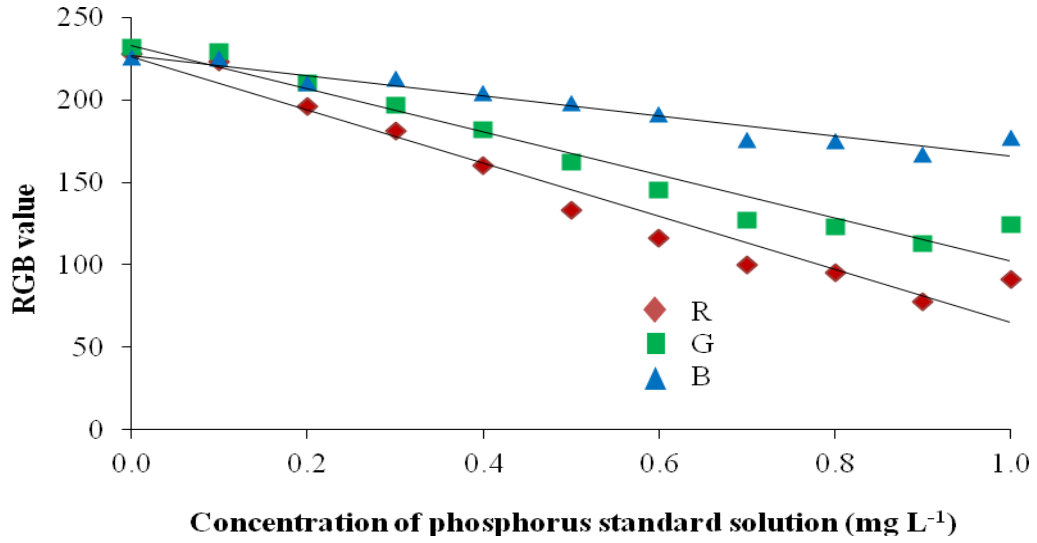

Figure 5. Plots of relationships between RGB values and concentration of phosphorus standard solution.

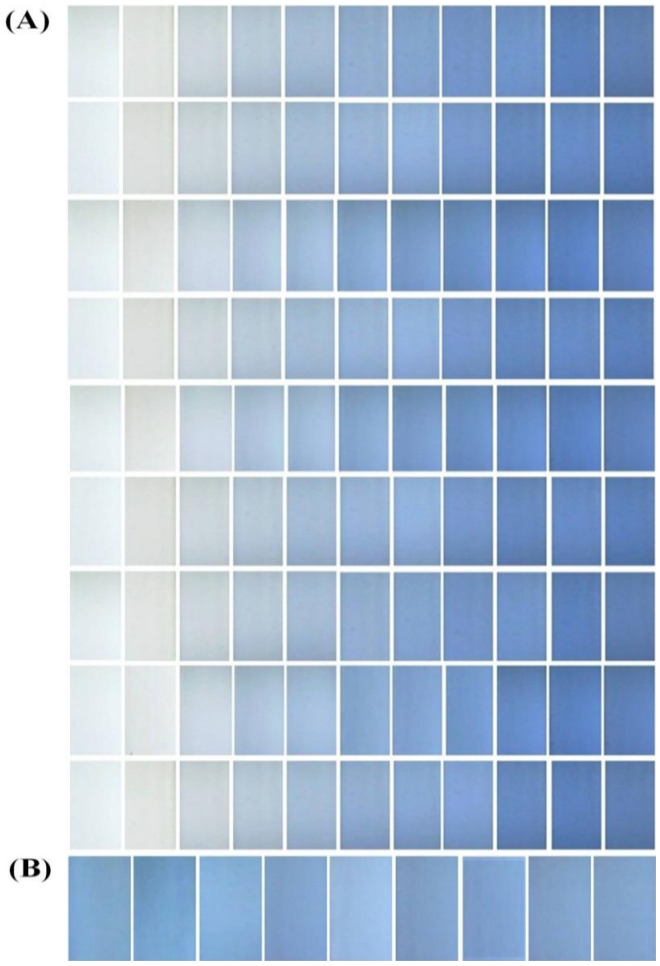

Figure 6. (A) Digital images of phosphorus standard solutions (0-1.0 mg L^{-1}) with molybdenum blue reagent (n=9) and (B) digital images of NR latex sample after digestion using UV digestion unit with molybdenum blue reagent (n=9).

program (only 5 min for data processing). The reproducibility of the phosphorus determination in NR latex was verified by the %RSD carried out between days for 11 replications. The %RSD of phosphorus standard solutions at 0.1, 0.3 and 0.5 mg L^{-1} were 3.72, 2.59 and 1.36%, respectively. This method showed good reproducibility for phosphorus determination at the concentration more than 0.1 mg L^{-1}. Moreover, the LOD could not be calculated because there was no calibration curve construction by the DIC-ANN method. The LOQ by the DIC-ANN defined as the concentration that could be photographed and processed by an ANN program was 0.1 mg L^{-1}.

Analysis of real samples

In this study, UV digestion unit was chosen for NR latex preparation before determination of total phosphorus by DIC-ANN compared with UV-Vis spectrophotometer. As illustrated in Figure 5, RGB value decreased with increasing phosphorus standard concentration whereas the intensity of color of the solution increased (Figure 6 A).The images of the complexes between phosphorus in NR latex with molybdenum blue solution after digestion were shown in Figure 6 B. The results from the determination of total phosphorus in NR latex samples after UV digestion were 0.170 ± 0.0181 mg g^{-1} (n=5) and 0.173 ± 0.0022mg g^{-1} (n=5) by DIC-ANN and UV-Vis spectrophotometry, respectively, which show no statistical difference at 95% confidence level by applying the paired t-test. These results do not exceed the level as compared to the DAP addition in NR latex recommended by Pollution Control Department (0.180 - 0.987 mg g^{-1} of phosphorus ion) (Pollution Control Department, 2005).

However, Karunanayake found that the phosphorus at aconcentration of more than 0.0098 mg g^{-1} would affect the stability of NR latex and the physical properties of products (Karunanayake and Perera, 2006).

Conclusions

The laboratory-made UV digestion unit used in combination with 30 g L^{-1} ammonium peroxodisulphate as oxidizing agent at 50 min digestion time was proved to be a successful NR latex preparation technique. It provided recovery ≥ 85% for phosphorus residue determination in NR latex samples using DIC-ANN and UV-Vis spectrophotometry. Therefore, it could be emphasized that the developed digestion procedure is simple, rapid, safety, accurate, precise and low cost (only USD 140) which are the main advantages. The UV digestion unit could be improved on increasing the number of sample per batch (more than 10 samples). Although, it could not be used continuously for the digestion in order to prevent the overheat in the UV digestion unit, it is still more interesting than the original technique such as Kjeldahl because using UV radiation assisted with thermal energy provides higher radical generating from oxidizing agent for decomposition reaction than using only thermal energy. Consequently, UV digestion unit is suitable for laboratory and small industrial factory in NR latex digestion before total phosphorus determination.

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