CRYOPRESERVATION OF SEEDS FOR LONG-TERM CONSERVATION OF COFFEE GERMPLASM AND ELITE VARIETIES : SUCCESSFUL APPLICATION AT CATIE

S. DUSSERT*, N. CHABRILLANGE*, N. VASQUEZ**, F. ANTHONY**, F. ENGELMANN*** S. HAMON*
*IRD, Genetrop, 911, av. d'Agropolis, BP 5045, 34032, Montpellier cedex 1, France
**CATIE, apartado 59, 7170 Turrialba, Costa Rica
***IPGRI, via delle Sette Chiese 142, 00145 Rome, Italy

Fonds Documentaire IRD
Cote : B* 22617 Ex : 1

Introduction

Though *C. arabica* seeds can withstand desiccation down to 0.06-0.08 g $H_2O.g^{-1}$ dw (Becwar *et al.* 1983 ; Ellis *et al.* 1990), they cannot be considered orthodox because they remain cold-sensitive and desiccation does not improve their longevity (Van der Vossen 1977; Ellis *et al.* 1990). *C. arabica* seeds are also characterized by their very short lifespan in the hydrated state (Couturon 1980).

Whatever their water content, *C. arabica* seeds do not withstand direct immersion in liquid nitrogen (Becwar *et al.* 1983). However, successful cryopreservation of zygotic embryos extracted from mature seeds has been achieved with *C. liberica* (Normah and Vengadasalam 1992), *C. arabica* (Abdelnour-Esquivel *et al.* 1992 ; Florin *et al.* 1993), *C. canephora* and the interspecific hybrid arabusta (Abdelnour-Esquivel *et al.* 1992). With all species tested, partial dehydration of excised embryos to 0.2 g H_2O/g dw was sufficient to obtain high survival after their direct immersion in LN.

Even if cryopreservation of excised zygotic embryos represents an interesting alternative strategy for long-term preservation of *C. arabica* genetic resources, this technique presents some drawbacks for routine use in coffee genebanks: i) in the case of coffee seeds, embryo extraction is very time consuming and labour intensive ; ii) low reproducibility was observed when desiccation zygotic embryos using classical desiccation methods (air-flow or silica gel); iii) all stages of the cryopreservation procedure have to be performed under aseptic conditions, which does not allow to avoid using *in vitro* culture techniques. Cryopreservation of whole seeds, instead of zygotic embryos, would allow to eliminate these drawbacks.

In this aim, the effects of several parameters of the cryopreservation protocol (desiccation, cooling, thawing and post-treatment) were investigated with *C. arabica* seeds to define conditions which would ensure survival of both the endosperm and the embryo (Dussert *et al.* 1997, 1998). It was shown that: i) the optimal water content for cryopreserving whole coffee seeds was 0.2 g H_2O/g dw; ii) a two-step freezing procedure including precooling at 1°C/min to -50°C was imperatively required to recover normal seedlings after cryopreservation; and iii) there was no effect of the thawing rate on survival of cryopreserved seeds. Under these conditions, the maximal percentage of normal seedlings produced after cryopreservation was about 30% (Dussert *et al.* 1997).In addition, it was observed that, whatever the cooling process, the survival rate of zygotic embryos extracted from cryopreserved seeds after thawing was always very high (80-90%). In this study, the reproducibility of seedling recovery was investigated by carrying out several cryopreservation experiments using the same optimal conditions. In addition, the effect of various post-thawing treatments was studied in order to improve the production of normal seedlings after cryopreservation of whole seeds.

CATIE coffee (*C. arabica*) collection is one of the most important in the world. This collection has been well characterized using molecular markers and agronomic traits. One of the objectives of CATIE is now to rationalize this collection in order to improve its long-

term conservation. Besides, excellent cryopreservation facilities are available in CATIE. Therefore, a collaborative project between CATIE and IRD, supported by IPGRI, was initiated in 1998 for the development of coffee seed cryopreservation techniques at CATIE. The aim of this project was to transfer to CATIE the methodologies for cryopreservation of coffee germplasm developed by IRD, to adapt them to local conditions and to test them on several accessions of the collection.

Materials and methods

Plant material, desiccation and cryopreservation

Fresh mature seeds of *C. arabica* var. Typica were employed. After the testa was removed, seeds were desiccated to 0.2 g H_2O/g dw by equilibration for 3 weeks under 78% RH obtained using a saturated NH_4Cl solution (Dussert *et al.* 1997). The different cryopreservation procedures (rapid cooling, two-step cooling, rapid rewarming) were carried out following the protocols described by Dussert *et al.* (1997, 1998).

Osmo-conditioning

After thawing, some seeds were osmo-conditioned for two weeks at 27°C in the dark using PEG solutions. Osmo-conditioning was carried out by placing batches of 10 seeds in Petri dishes sealed with Parafilm Ribbon, on a thin layer of cotton wool imbibed with 20 ml of aqueous PEG 6000 solution. PEG concentrations were calculated to achieve osmotic potentials of -1, -2 and -4 MPa at 27°C using the equation given by Michel and Kaufmann (1973).

Culture conditions

After thawing and/or osmo-conditioning, seeds were cultured *in vitro* for survival assessment. Disinfection and *in vitro* culture were performed as described by Dussert *et al.* (1997). Extraction of zygotic embryos and *in vitro* culture were carried out according to the method of Bertrand-Desbrunais and Charrier (1989).

Survival assessment

Both germination *sensu stricto* and development of normal seedlings were used to assess seed survival. Emergence of the hypocotyl and radicle was used as the criterion for estimating the germination rate. Seedlings which stood upright on the medium were considered normal. Excised embryos were considered viable when they stood upright on the culture medium and when their first pair of leaves was developed. The time to reach half of the final proportion (P_f) of normal seedlings, T_{50}, was estimated using the least square regression and the following model where P is the proportion of normal seedlings, T the time in days and A a treatment-dependant variable describing the synchronisation of seedlings development : $P = P_f / (1 + \exp(A (T - T_{50})))$.

Test at CATIE of cryopreservation procedures set up at IRD

In order to verify the reproducibility of results obtained at IRD (France), two cryopreservation procedures resulting from the experiments described above were performed at CATIE using *C. arabica* var. Typica seeds. In both procedures, seeds were desiccated to 0.2 g $H_2O.g^{-1}$ dw by equilibration for 2 weeks over a NH_4Cl saturated salt solution. In procedure 1, seeds were cooled rapidly (direct immersion into LN) and thawed rapidly in a 40°C water-bath for 3 min before zygotic embryos were extracted from cryopreserved seeds and germinated *in vitro*. In procedure 2, seeds were cooled slowly by precooling them at 1°C/min to -50°C prior to immersion into LN, thawed rapidly and germinated *in vitro*.

Table 1. Number of experiments (n) and percentage (mean, minimal and maximal values) of normal seedlings recovered from whole seeds or extracted zygotic embryos after desiccation of seeds to 0.2 g H_2O/g dw, followed by rapid cooling (direct immersion in LN; 200°C/min) or slow cooling (1°C/min to -50°C prior to immersion in LN).

	Whole seeds		Zygotic embryos	
	n	Seedlings (%) (min-max)	n	Seedlings (%) (min-max)
Rapid cooling	4	0	3	91 (83-98)
Slow cooling	7	17 (6-29)	1	70

Results

Effects of slow and rapid cooling

When cooled rapidly (200°C/min) by direct immersion in LN, none of the cryopreserved seeds produced normal seedlings. By contrast, viability of zygotic embryos extracted from rapidly cooled seeds was always very high. If seeds were precooled to -50°C at 1°C/min before immersion in LN (slow cooling), over 7 repeats, a mean value of 17% of cryopreserved seeds developed into normal seedlings. Under these conditions, some normal seedlings were always recovered after germination of whole seeds, but high variability in the final survival rate was observed (6 to 29%).

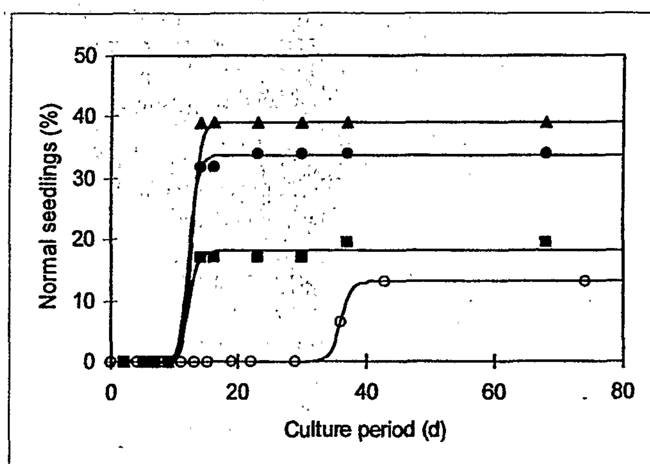

Figure 1. Evolution with time in culture under germination conditions of the percentage of normal seedlings recovered from cryopreserved seeds after a 2 weeks osmo-conditioning treatment on PEG solutions at -1 (▲), -2 (●) and -4 (■) MPa or without osmo-conditioning treatment (O).

Effect of osmo-conditioning of seeds after rewarming

Osmo-conditioning of seeds after rewarming improved the final proportion of normal seedlings recovered from cryopreserved seeds but the gain in survival decreased in line

with decreasing osmotic potential of the PEG solution used for osmo-conditioning treatment (Fig. 1). Production of normal seedlings under optimal osmo-conditioning conditions (-1 MPa) was three-fold higher than that of non osmo-conditioned cryopreserved seeds. Post-thawing seed osmo-conditioning drastically reduced T_{50}, the time to reach half of the final proportion of seedlings: when cryopreserved seeds were placed under germination conditions directly after thawing, T_{50} value was 36 d, while with osmo-conditioned seeds, it was about 12-13 d, independently of the osmotic potential of the osmo-conditioning solution.

Test at CATIE of cryopreservation procedures set up at IRD

Desiccation to 0.2 g H_2O.g^{-1} dw by equilibration over a saturated NH_4Cl solution had no detrimental effect on seed viability since 100% of desiccated seeds developed into normal seedlings.

One hundred percents of embryos extracted from cryopreserved seeds according to the Procedure 1 (desiccation to 0.2 g H_2O.g^{-1} dw, rapid cooling and rapid thawing) developed into normal seedlings. These seedlings showed a normal development after transfer under greenhouse conditions

All seeds cooled slowly and placed under germination conditions directly after rewarming (Procedure 2) showed hypocotyl extrusion and radicle growth. Further development into normal seedlings occurred in 24% of cryopreserved seeds. This final percentage of normal seedlings is very similar to those observed at IRD over 7 repeats (Table 1).

Discussion

When seeds of *C. arabica* at 0.2 g H_2O/g dw were cooled rapidly (200°C/min), none of them developed into normal seedlings. This result is consistent with those of Becwar *et al.* (1983) who showed that *C. arabica* seeds did not survive after immersion in LN, even if all freezable water had been removed from the seeds. By contrast, when seeds were slowly precooled to -50°C at 1°C/min before immersion in LN, an average value of 17% (over 7 repeats) of cryopreserved seeds developed into normal seedlings. It is thus clear that slow precooling of *C. arabica* seeds had a dramatic effect on their survival and their capacity to develop normally (Dussert *et al.* 1998). However, in view of the high variability observed for the survival rate over 7 experiments, improvement to the method appeared necessary before routine use and various post-thawing treatments were investigated.

It was shown for the first time that seed osmo-conditioning (osmo-conditioning) carried out after thawing had a dramatic beneficial effect on the proportion of normal seedlings recovered after cryopreservation and on seedling growth rate: under optimal osmo-conditioning conditions (-1 Mpa for 2 weeks), the percentage of cryopreserved coffee seeds which developed into normal seedlings was three-fold that of non osmo-conditioned cryopreserved seeds (39 % *vs* 13%) and the time to reach half of final percentage of normal seedlings (T_{50}) was about three-fold lower (13 *versus* 36 d). To our knowledge, the effect of seed osmo-conditioning after cryopreservation has been investigated previously in one study on celery seeds only (Gonzales-Benito *et al.* 1995). In this study, no effect of osmo-conditioning could be found since cryopreservation did not affect germination rates and T_{50} values. Seed osmo-conditioning was first employed to improve their rate and uniformity of germination (Heydecker *et al.* 1975). Since then, a beneficial effect of osmo-conditioning after seed ageing has been observed both on germination percentage and germination rate with numerous species (Bewley and Black 1994; Bray 1995).

Thus, even if the percentage of seeds which developed into normal seedlings remained relatively low in comparison with that obtained from excised zygotic embryos, a combination of slow cooling and osmo-conditioning treatment could represent a simple and

efficient complementary option to field conservation for genebanks which cannot afford *in vitro* culture facilities. Moreover, this method might be simplified by using a -80°C freezer for precooling seeds to -50°C and could thus become more easily employed routinely in a larger number of genebanks maintaining coffee genetic resources. However, additional research should be undertaken to optimize osmo-conditioning conditions and to carry out direct germination tests under greenhouse or nursery conditions.

In cases where very high survival rates are required for routine use, we propose a second alternative approach based on the extraction of zygotic embryos after rewarming of seeds, which would allow to avoid most of the drawbacks of cryopreservation protocols developed for zygotic embryos. Equilibrating coffee seeds under 78% RH allowed seeds to reach optimal water content for cryopreservation in a very easy and reproducible manner. This method also allows the processing of large amounts of seeds at the same time. Moreover, aseptic culture conditions are requested after thawing only.

Both procedures proposed in this study were successfully applied at CATIE. Optimization of post-thawing osmo-conditioning conditions and testing of the two procedures on 14 accessions of *C. arabica* are currently under investigation at CATIE. Some preliminary results indicate that up to 70% of normal seedlings could be recovered from cryopreserved seeds (without zygotic embryo extraction) under some osmo-conditioning conditions. Testing of optimized protocols on 14 accessions of *C. arabica* will provide preliminary information on the feasibility of establishing a *C. arabica* germplasm cryobank at CATIE.

Abbreviations

CATIE : Centro Agronomico Tropical de Investigacion y Enseñanza ; dw : dry weight ; LN : liquid nitrogen ; IRD : Institut de recherche pour le développement ; IPGRI : International Plant Genetic Resources Institute.

References

Abdelnour-Esquivel, A., V. Villalobos and F. Engelmann. 1992 Cryopreservation of zygotic embryos of *Coffea* spp. *Cryo-Letters* 13: 297-302.

Becwar, M.R., P.C. Stanwood and K.W. Lehonardt. 1983. Dehydration effects on freezing characteristics and survival in liquid nitrogen of desiccation-tolerant and desiccation-sensitive seeds. *Journal of the American Society of Horticultural Science* 108: 613-618.

Bertrand-Desbrunais, A. and A. Charrier. 1989. Conservation des ressources génétiques caféières en vitrothèque. Pp. 438-447 *in* Proceedings of the 13th ASIC, Paipa, Colombia.

Bewley, J.D. and M. Black (eds.). 1994 Seeds. Physiology of Development and Germination. 2nd Edition. Plenum Press, New York.

Bray, C. 1995. Biochemical processes during the osmo-conditioning of seeds. Pp. 767-780 *in Seed development and germination* (J. Kigel and G. Galili, eds.). Marcel Dekker Inc., New York.

Couturon, E. 1980. Le maintien de la viabilité des graines de caféiers par le contrôle de leur teneur en eau et de la température de stockage. *Café Cacao Thé* 1: 27-32.

Dussert, S., N. Chabrillange, F. Engelmann, F. Anthony and S. Hamon. 1997. Cryopreservation of coffee (*Coffea arabica* L.) seeds: importance of the precooling temperature. *Cryo-Letters* 18: 269-276.

Dussert, S., N. Chabrillange, F. Engelmann, F. Anthony, J. Louarn and S. Hamon. 1998. Cryopreservation of seeds of four coffee species (*Coffea arabica, C. costatifructa, C. racemosa* and *C. sessiliflora*): importance of water content and cooling rate. *Seed Science Research* 8: 9-15.

Ellis, R.H., T.D. Hong and E.H. Roberts. 1990. An intermediate category of seed storage behaviour ? I. Coffee. *Journal of Experimental Botany* **41**: 1167-1174.

Florin, B., H. Tessereau and V. Pétiard. 1993. Conservation à long terme des ressources génétiques de caféier par cryoconservation d'embryons zygotiques et somatiques et de cultures embryogènes. Pp. 106-113 *in* Proceedings of the 15th ASIC, Montpellier, France.

Gonzales- Benito, M.E., J.M. Iriondo, J.M. Pita and F. Perez-Garcia. 1995. Effect of seed cryopreservation and priming on germination in several cultivars of *Apium graveolens*. *Annals of Botany* **75**: 1-4.

Heydecker, W., J. Higgins and Y.J. Turner. 1975. Invigoration of seeds. *Seed Science & Technology* **3**: 881-888.

Michel, B.E. and M.R. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiology* **51**: 914-916.

Normah, M.N. and M. Vengadasalam. 1992. Effects of moisture content on cryopreservation of *Coffea* and *Vigna* seeds and embryos. *Cryo-Letters* **13**, 199-208.

Van der Vossen, H.A.M. 1977. Methods of preserving the viability of coffee seed in storage. *Kenya Coffee* **45**: 31-35.

Association Scientifique Internationale du Café

18ème

COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ

18th

INTERNATIONAL SCIENTIFIC COLLOQUIUM ON COFFEE

18.

INTERNATIONALES WISSENSCHAFTLICHES KOLLOQUIUM ÜBER KAFFEE

18°

COLÓQUIO CIENTÍFICO INTERNACIONAL SOBRE EL CAFÉ

Helsinki, 2-6 août 1999

ISBN 2-900212-17-0

DIX-HUITIEME COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFE

Helsinki, 2 - 8 août 1999

Association Scientifique Internationale du Café (ASIC)
Siège social : 18, rue de la Pépinière, 75008 PARIS

DIX-HUITIÈME COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ
Helsinki, 2-6 août 1999

Introduction

- Universities and Companies in collaboration, Risto Ihamuotila .. 9
- New challenges for the coffee sector, Bertel Paulig 13

Communications et posters

Chimie

Communications

- Key odorants of roasted coffee : evaluation, release, formation, W. Grosch 17
- Evolution of coffee aroma characteristics during roasting, C. Gretsch *et al*.......................... 27
- Differences in chemical composition of electronically sorted green coffee beans, G. Full *et al*. 35
- Characterisation of mouldy / earthy defect in green Mexican coffee, E. Cantergiani *et al*. 43
- The detection and characterisation of free radicals generated during the decomposition of solutions of the coffee flavour compound furfuryl mercaptan, E.C. Pascual *et al*. ... 50
- Retardation of coffee beverage volatiles by different milk products, H. Steinhart, M. Bücking 58
- Diterpene degradation products in roasted coffees, K. Speer *et al*. 65
- Carboxyatractyligenin and atractyligenin glycosides in coffee, A.G.W. Bradbury, H.H. Balzer 71
- Stable isotopes and coffee quality: preliminary report, F. Serra *et al*. 78
- Nonvolatile compounds in coffee, S. Homma.................................. 83
- Structural aspects of polysaccharides from Arabica coffee, M. Fischer *et al*. 91
- Isolation and characterisation of a foaming fraction from hot water extracts of roasted coffee, M. Petracco *et al*....................... 95

Posters

- Effects of saliva and milk additives on the coffee flavour release in the oral cavity, M. Bücking *et al*. 106
- Evaluation of acidity and bitterness of coffee brew, K. Aino, M. Motoyoshi.................. 110
- A new method for the determination of 16-O-Methylcafestol in roasted coffee, I. Kölling-Speer *et al*. 114
- Determination of the relationship between phosphate concentration and perceived acidity in coffee, M.J. Griffin, D.N. Blauch 118

Génie alimentaire

Communications

- Structural properties of coffee beans as influenced by roasting conditions, S. Schenker *et al*. 127
- Online analysis of food processing gases by resonance laser mass spectrometry (RREMPI-TOFMS) : coffee roasting and related applications, R. Dorfner *et al*. 136
- Evaluation of a microwave sensor for inline measurement of roasted and green whole coffee bean moisture, G.A. Wiseman ... 143
- The relation between volatile retention and movement of ice front during freeze drying of coffee, J.M. Pardo *et al*. 150
- Kinetics of coffee infusion : determination of the geographical origin of medium roasted coffees using extraction kinetics, D. Jaganyi.................... 159

– Decaffeination of non-aqueous solvents using caffeic acid, B.L. Zeller, F.Z. Saleeb 168

Posters

– Computer simulation as a tool to model coffee brewing cellular automata for percolation processes ; 2D and 3D techniques for fluid-dynamic simulations, R. Cappuccio, F. Suggi Liverani 173

– Mapping of Brazilian coffee quality, E.E.M. Mori *et al.* 179

Effets physiologiques

Communications

– A new physiological method to evaluate gastric irritation of different coffees, P.W. Lücker.... 185

– Biotransformation and lack of mutagenicity of ochratoxin A using combinations of mammalian biotransformation enzymes, H. Zepnick *et al.* 193

Workshop I

– Workshop report : effects of coffee on brain and behaviour. What does coffee consumption bring in daily life ? A. Nehlig.... 201

- Behavioral effects of caffeine in coffee, A. Smith, C. Brice........ 204
- Effects of caffeine on attention, J. Snel *et al.* 208
- Effects of coffee and caffeine on memory and aging, M.P.J. van Boxtel *et al.* 209
- Neurochemical effects of caffeine and behavioural tolerance, B.B. Fredholm *et al.* 212
- Reinforcing effect of caffeine, E. Zvartau 213
- Caffeine does not activate the brain structures involved in drug addiction, A. Nehlig........ 215

Workshop II

– Report on the workshop "Enhancement of coffee quality by reduction of mould growth", G. van der Stegen, M. Blanc...... 219

- Modelling and HACCP tools for coffee quality improvement, J.M. Frank.............................. 223
- Fungi associated with the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera : Scolytidae), F.E. Vega *et al.* 229
- Fungi producing ochratoxin A in coffee, M.H. Taniwaki *et al.* 239
- Recent international developments in the field of mycotoxins, E. Boutrif............ 248
- ICO/FAO/CFC project : enhancement of coffee quality through prevention of mould formation, E. Boutrif.............. 257
- First Uganda FAO mission - How near is an HACCP system in dry Robusta coffee production ? R. Viani 258
- Enhancement of quality in coffee by prevention of mould formation project, C.P.R. Dubois.................................... 260
- Studies on ochratoxin A in Indian coffees and its management strategies, R. Naidu *et al.* 261
- Enhancement of coffee quality through the prevention of mould growth : start up experiences in Uganda, H. Ngabirano 265
- Levels of ochratoxin A in blood from Norwegian and Swedish blood donors : estimated intakes and correlation between blood levels and food consumption habits, A. Thuvander *et al.* 276

Agronomie

Biolotechnologie

Communications

– Potential, progress and future thrust areas of coffee biotechnology research in India, H.L. Sreenath, R. Naidu............ 281

– A technically and economically attractive way to propagate elite *Coffea canephora* (Robusta) clones : *in vitro* somatic embryogenesis, J.P. Ducos *et al.* 295
– Coffee tissue culture as a new model for the study of somaclonal variation, V.M. Loyola-Vargas *et al.* 302
– Cryopreservation of seeds for long-term conservation of coffee germplasm and elite varieties : successful application at CATIE, S. Dussert *et al.* 308
– Molecular characterisation of the cultivar Bourbon L.C., S. Zezlina *et al.* 314
– Cloning and characterisation of fruit-expressed ACC synthase and ACC oxydase from coffee, K.R. Neupane *et al.* 322
– Cloning and characterisation of xanthosine-N7-methyltransferase the first enzyme of the caffeine biosynthetic pathway, S. Moisyadi *et al.* 327
– Genetically modified coffee trees for resistance to coffee leaf miner. Analysis of gene expression, resistance to insects and agronomic value, T. Leroy *et al.* 332
– *In vitro* culture of immature embryos of *Coffea arabica* cv Catimor, M.C. Simões-Costa *et al.* 339

Poster

– Polymorphic microsatellites in *Coffea arabica*, R. Mettulio *et al.* 344

Sélection et amélioration génétique

Communications

– Première évaluation d'hybrides naturels entre *Coffea canephora* et *Coffea arabica* de Nouvelle-Calédonie, P. Jagoret *et al.* 349
– Creation and selection of *Coffea arabica* hybrids in Tanzania, N.E. Nyange *et al.* 356
– Etude des paramètres génétiques de la production et de caractères associés à partir d'un plan de croisement diallèle chez *Coffea arabica*, C. Cilas *et al.* 363
– Evaluation of an advanced breeding population of Arabica coffee, C.O. Omondi *et al.* 371
– Flavour : an ideal selection criterion for the genetic improvement of liquor quality in Arabica coffee, C.O. Agwanda... 383

Posters

– Increasing Robusta production in Brazil. The potential of 200 000 hectares in São Paulo State, H.P. Medina-Filho *et al.* 390
– Coffee cultivars in Brazil, L.C. Fazuoli *et al.* 396
– Twenty seven years of coffee breeding in Kenya : prospects for the release of new varieties, C.O. Agwanda 405

Pratiques agronomiques

Communications

– The influence of available water on crop development and yield of coffee (*Coffea arabica* L.) at Aiyura, Papua-New-Guinea, J.V. Enden, P.H. Hombunaka............ 407
– Small holder coffee irrigation research in Kenya, M.P.H. Gathaara.................................... 415
– Response of clonal Robusta coffee to organic and mineral fertiliser application in lake Victoria crescent zone, R.J. Onzima *et al.* 418
– The effect of green manure application to coffee plants growth, yield and quality in Kenya, J.K. Kimemia *et al.* 426
– Mise en évidence du transfert d'azote des légumineuses aux caféiers par l'utilisation des méthodes isotopiques, D. Snoeck, A.M. Domenach 430

Posters

- Evaluation of some leguminous species for the establishment of Robusta coffee in Ghana, K. Osei-Bonsu *et al.* 438
- Preliminary investigations into the use of intercropping for weed management in young coffee in Ghana, K. Opoku-Ameyaw *et al.* 441
- Coffee pruning and spacing - management of tall Arabica coffee (*Coffea arabica* L.) in Papua-New-Guinea, P. Talopa, J-M. Kiara 445
- Use of humic acid in promoting growth of young coffee Robusta seedlings in Nigeria, C.R. Obatolu...................................... 449
- Prediction of yield stability in Arabica coffee based on the stability of morphological components, C.O.Agwanda *et al.* 452
- Towards efficient coffee marketing in Kenya, M.T. Osongo 454

Technologie après-récolte

- How to avoid mould troubles in green coffee preparation, A.A. Teixeira 457
- Cell wall polysaccharides of coffee bean mucilage. Histological characterisation during fermentation, S. Avallone *et al.* 463

Maladies et insectes

Communications

- Effet de différentes conditions agro-écologiques sur le développement de l'anthracnose des baies du caféier Arabica dans l'ouest du Cameroun. Incidence sur le mode de conduite des plantations et l'application des traitements phytosanitaires, D. Bieysse *et al.*.............................. 471
- Histochemical differences during infection of *Coffea arabica* varieties by *Colletotrichum kahawae* isolates, E.K. Gichuru *et al.* 477
- Piégeage de masse du scolyte du café *Hypothenemus hampei* Ferr. (Col., Scolytidae) en conditions réelles : premiers résultats, B. Dufour *et al.* 480
- The role of parasites in the natural control of Antestia, *Anstestiopsis intricata* (Ghesquière and Carayon). Possibilities for further control using exotic parasites, M. Abebe 492

Posters

- Pre-selection methods for coffee berry disease resistance in Ethiopia, E. Derso 497
- Proteolytic enzyme activity in *Coffea arabica* varieties varying in resistance to coffee berry disease, E.K. Gichuru, P.N. Kingiori.................................... 504
- Techniques for screening resistance to coffee berry disease (*Colletotrichum kahawae* Waller & Bridge), D. Kilambo *et al.* 508
- Evaluation en champ de l'efficacité de certaines formulations de fongicides vis-à-vis de l'anthracnose des baies du caféier Arabica, J. Bakala, E. Nyemb...................................... 512
- Method of rearing larvae and some aspects of the biology and control of cocoa stem borer *Eulophonotus myrmeleon* (Felder), M. Abebe.................... 517
- Caffeine does not protect coffee against the leaf miner *Perileucoptera coffeella*, O. Guerreiro Filho, P. Mazzafera.... 520
- Insects associated with coffee berries in Ghana, B. Padi.......... 524

Rapports de synthèse / Summary reports.......................... 529

Participants 549