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ANTIULCER EFFECTS OF AQUEOUS EXTRACT AND A FRACTION OF *PHYLLANTHUS EMBLICA* FRUIT ON GASTRIC ACID SECRETION AND MUCOSAL DEFENCE FACTORS IN ALBINO RATS

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Abstract: *Phyllanthus emblica* (Euphorbiaceae) fruit has been empirically used since centuries in folkloric medicine to treat gastrointestinal disorders including the gastric ulcers. In the present study, anti-ulcerogenic properties of the fruit, its aqueous extract and a purified fraction were determined in albino rats. Aqueous extract of the fruit protected rats against gastric ulcers induced by indomethacin. Partition of the water extract yielded fractions for which anti-ulcerogenic activity evaluation studies were conducted to find out the most effective fraction. Thin layer chromatography yielded the most purified active fraction, which was found to exert anti-ulcerogenic activity in the chemically induced and stress-induced gastric ulcers in albino rats. In addition, effect of the purified fraction on gastric secretion volume, pH, acid output, ulcer index, mucus secretion and peptic activity revealed it to be the most potent anti-ulcer fraction with efficacy comparable to the reference drug, famotidine. It may be suggested that anti-ulcerogenic activities of *P. emblica* fruit, Its aqueous extract and the purified fraction could be due to elevation of gastric mucus secretion and inhibition of gastric acid secretion.

Keywords: Antiulcer effects, aqueous extract, *Phyllanthus emblica*, stomach, albino rats

Introduction

Plants have been used since centuries for the treatment of various diseases [1,2]. The increased awareness to untoward effects of allopathic drugs has encouraged the scientists to look for alternative drugs. *Phyllanthus emblica* (Euphorbiaceae) fruit has been considered useful in both acid-peptic ulcer and non-ulcer dyspepsia [3,4]. Its fruits have been used as carminative, tonic as well as antipyretic, and have also been prescribed in certain heart and liver complaints and in urinary disorders by the practioners of folk medicine. In addition, the fruit has been considered in traditional medicine as one of the best drug imparting a healthy long life. The dried fruit powder is applied to hair and skin for increasing hair growth and luster [5,6,7]. Furthermore, presence of ascorbic acid, tannins, saponins, lipase corilagin, hormones like gibberellin A-1, A-3, A-4, A-7 and A-9, alkaloids such as zeatin nucleotide, zeatin riboside and astragalin (a flavonoid) have been reported in this fruit [8,9,10]. Moreover, crude plant products have been generally found to be safer and relatively free from side effects [11].

In the present study, antiulcerogenic properties of *Phyllanthus emblica* fruit, its aqueous extract and purified fraction in albino rats have been investigated.

Materials and Methods

Plant Material and Chemical Processing

Phylanthus emblica fruit, locally called as Amla, was obtained in bulk amount from a leading herbal dealer of Faisalabad, Pakistan. The plant material was authenticated and compared with its respective standard in the herbarium maintained by

the Department of Botany, University of Agriculture, Faisalabad. Its sample is preserved in the Pharmacognosy Laboratory, Department of Pharmacy, Islamia University Bahawalpur. The plant material to be used was further dried under-the-shade, finely powdered and extracted in water at 37ºC [12,13]. Aqueous extract was divided into butanol, chloroform and ethyl acetate soluble fractions [14,15]. Thin layer chromatography on silica gel G (10-40 µm) plates, eluted with ethyl acetate:water (53:47), was used to follow purification of ethyl acetate fraction [16]. The plates were first examined under short UV light and then developed either with ninhydrin, iodine, vanillin or 1% ferric chloride solution. Purified entities of aqueous-ethyl acetate fraction were obtained by column chromatography with the same solvent system i.e. aqueous ethyl acetate 47 fraction [17,18].

Animals

Adult healthy Sprague-Dawley albino male rats, weighing 100-200g each, were obtained from University of Agriculture, Faisalabad. The animals were housed under conditions of 23 ± 12ºC temperature, 55 ± 15% humidity and 12 h light (7.00-19.00), as used by Sorba *et al*. [19]. These rats were provided with free access to the standard feed (M/S Lever Brothers, Rahim Yar Khan) and water *ad libitum*. The rats were fasted for 24 h prior to their use in the experiments [20,21].

Grouping of Animals

Animals were randomly divided into 17 groups of 6 rats each, except for the untreated control groups in which there were 9 rats each. This latter group received 5 ml/kg of 1% tragacanth vehicle twice daily, alongwith the indomethacin (25 mg/kg). As reference controls, 6 rats were treated orally with aqueous extract of *P. emblica* fruit (eq. to 15 g/kg), aqueous ethyl acetate and aqueous ethyl acetate 47 fractions (eq. to 1.5 g/kg) of powdered *P. emblica* fruit twice daily. Similar groups were set up for evaluation of the fruit's extract/fractions in histamine and hypothermia restraint-induced ulcerated rats.

Gastric Secretion Volume, pH and Acid Output

The rat stomachs were excised under ether anesthesia 4 h after pylorus ligation for collection of gastric contents. The contents were centrifuged at 4000 rpm for 10 minutes and the supernatants were collected separately [22]. The volume and pH of all supernatants were measured and their acidities were determined by titration to pH 7 with 0.1N sodium hydroxide solution [22,23]. The acid outputs were calculated according to the method of Ishizuka *et al*. [21].

Gastric Ulcer Index

All stomachs were examined under a light microscope. The damage to the gastric mucosa of the glandular regions appeared as elongated black-red lesions running parallel to the long axis of the stomachs. The length (mm) of each lesion was measured and gastric ulcer index was calculated by adding the length of all lesions in the fundic region [24].

Gastric Pepsin Activity

Gastric pepsin activity was determined by using the centrifuged gastric juice, with bovine albumin as substrate [25,26].

Gastric Mucus Content

Using the method of Corne *et al*. [27], the glandular portions of the stomachs were collected from all experimental animals and were immediately transferred to 1% alcian blue solution in 10% sucrose. Thus, the glandular mucus was allowed to complex with alcian blue for 30 min, which was extracted for 15 min in 5 ml of 5% magnesium chloride solution. The solution was then shaken with equal volume of diethyl ether and the emulsion was centrifuged at 4000 rpm for 15 min. The aqueous layer was read at 580

Statistical Analysis

Mean values (± SEM) for various parameters for rats of each group were computed. In order to see the magnitude of variation between groups, the data were analyzed statistically using t-test [28].

Results

Aqueous Extract of P. emblica Fruit

As shown in Table 1, oral administrations of aqueous extract equivalent to 1.5 g/kg of the powdered *P. emblica* fruit reduced the gastric juice volume and acid output, while it significantly ($P<0.001$) increased the gastric pH in indomethacin-treated rats. The extract also highly significantly inhibited the formation of gastric lesions (i.e. ulcer index) in the treated animals.

Purified Fraction of P. emblica Fruit

Table 2 shows that *P. emblica* aqueous-ethyl acetate 47 fraction (PE-AqA 47) significantly ($P<0.001$) inhibited the increase in the gastric juice volume, acid-output, pepsin activity, and lesion formation induced by indomethacin. PE-AqA 47 also antagonized highly significantly ($P<0.001$) the decrease in gastric secretion, pH and mucus secretion in the treated rats. The reference drug famotidine caused significant ($P<0.05$) effect on gastric juice volume and mucus secretion while highly significant ($P<0.001$) preventive effect on gastric pH, acid-output, pepsin activity and ulcer index.

Table 2 also shows that PE-AqA 47 did not change the gastric juice volume, pH, acid output and pepsin activity in the histamine-treated rats. However, it blocked highly significantly ($P<0.001$) gastric lesion formation and increased mucus secretion. Famotidine also antagonized highly significantly ($P<0.001$) the decrease in gastric secretion, pH, increase in gastric acid output and lesion formations induced by histamine. Table 3 shows that both PE-AqA 47 and famotidine inhibited highly significantly ($P<0.001$) gastric lesion formation in the hypothermic-restrained rats. It is worth mentioning that the purified fraction was more effective ($P<0.001$) in increasing mucus secretion in the treated rats than was the reference drug ($P<0.05$) in the treated rats.

Discussion

Table 1. Effects of *P. emblica* fruit powder and its aqueous extract on gastric secretion volume, pH, acid-output and ulcer index in rats.

Treatment	Gastric Secretion			Ulcer Index (mm)
	Volume (ml) Mean±S.E.M.	pH Mean±S.E.M.	Acid Output (µEq/100g/4hr) Mean±S.E.M.	Mean±S.E.M.
Normal (Untreated control)	2.20 ± 0.06	2.82 ± 0.01	2.82 ± 0.15	2.62±0.3
Indomethacin (25mg/kg p.o) Treated Control	8.6 ± 0.45 **	1.08 ± 0.20**	340.23±14.21**	49.83± 2.47**
Aqueous extract of P. emblica fruit (Eq. to 1.5 g/kg p.o.) + Indomethacin (25 mg/kg s.c.)	2.8 ± 0.07**	3.18 ± 0.06**	1.65 ± 0.17**	5.5±0.3**

Indomethacin: Significant from untreated control (Normal) * $P<0.05$; ** $P<0.001$
Test drugs: significant from treated control (Indomethacin) * $P<0.05$; * $P<0.001$
Standard error of means (S.E.M) of six experiments.

Table 2. Antiulcer activities of purified fractions of *P. emblica* fruit powder in indomethacin and histamine-ulcerated rats.

Experimental Procedures	Treatment	Gastric Secretion			Ulcer Index (mm)	Mucus Secretion (mg/hr)	Pepsin Activity (mg/hr)
		Volume (ml) Mean ± S.E.M	pH Mean ± S.E.M	Acid-output Eq/100 g/4 hr) Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
Indomethacin treated	Indomethacin (25mg/kg *s.c.*) (Treated Control)	8.6 ± 0.45	1.08 ± 0.20	340.23± 14.21	49.83 ± 2.47	0.203 □ 0.02	21.53 □ 0.91
	Famotidine (100 mg/kg *p.o.*) + Indomethacin (25mg/kg *s.c.*)	7.27 ± 0.24*	2.89 ± 0.30**	9.18 ± 1.23**	10.83 ± 1.20**	0.244 □ 0.01*	16.11□ 0.02**
	P. emblica aqueous-ethyl acetate fraction (Eq. to 1.5 g/kg *p.o.*) + Indomethacin (25 mg/kg *s.c.*)	2.40 ± 0.06**	3.48 ± 0.25**	0.66 ± 0.15**	6.00 ± 0.79**	0.285 □ 0.01**	6.81□ 0.72**
	P. emblica aqueous-ethyl acetate 47 (Eq. to 1.5 g/kg *p.o.*) + Indomethacin (25 mg/kg *s.c.*)	2.15 ± 0.07**	3.92 ± 0.36**	0.26 ± 0.09**	3.42 ± 0.39**	0.309 □ 0.02**	2.47□ 0.80**
Histamine treated	Histamine (25mg/kg *p.o*) (Treated Control)	6.0 ± 0.28	1.04 ± 0.11	310.91± 9.25	61.83 ± 1.94	0.212 □ 0.01	23.63 □ 0.76
	Famotidine (100 mg/kg *p.o.*) + Histamine (25mg/kg *s.c.*)	5.27 ± 0.43	2.89 ± 0.30**	5.66 ± 0.23**	8.83 ± 1.17**	0.234 □ 0.01	16.71□ 0.12**
	P. emblica aqueous-ethyl acetate fraction (Eq. to 1.5g/kg *p.o.*) + Histamine (25mg/kg *s.c.*)	5.82 ± 0.15	1.22 □ 0.12	289.83 ± 9.90	38.84 ± 2.68	0.259 □ 0.01**	21.13 □ 0.54
	P. emblica aqueous-ethyl acetate 47 (Eq. to 1.5g/kg *p.o.*) + Histamine (25mg/kg *s.c.*)	5.41 ± 0.49	1.19 ± 0.31	291.08 ± 6.28	30.48 ± 1.56**	0.295 □ 0.02**	21.83 □ 0.61

Test drugs: significant from treated control (Histamine) * $P < 0.05$; ** $P < 0.001$
All the other values are N.S. ($P > 0.05$) from treated control (Histamine).
S.E.M = Standard error of means of six experiment

Peptic ulcers are caused when the natural balance between the aggressive factors of acid and pepsin and defensive mechanism of mucus, bicarbonate, mucosal turnover and blood supply (mucosal barrier) are disturbed. Because of this varied ulcer pathogenesis, various types of antiulcer drugs have been used to either overcome the acid and pepsin secretion (e.g. antacids and H_2-blocking drugs) or to enhance mucus secretion to stabilize the surface epithelial cells (e.g. sucralfate, which binds to the proteinaceous material in the ulcer crater and prevents further digestion of the mucosa by acid and pepsin [29]). These drugs have decreased morbidity and mortality but may also produce many adverse reactions such as arrhythmias, impotence, gynaecomastia, haematopoietic changes and high recurrence rates [30].

In the present study, the antiulcerogenic efficacy of the test plant was determined in rats having indomethacin, histamine and stress-induced ulcers. The data obtained showed that aqueous extract of *P. emblica* fruit (PE), its aqueous-ethyl acetate (PE-AqA) fraction and the purified fraction PE-AqA 47 significantly ($P<0.001$) antagonized the indomethacin-induced effects on gastric secretion volume, pH, acid-output and ulcer index. Additionally, PE-AqA and PE-AqA 47 also inhibited the effects on pepsin activity and mucus secretion. Among these, PE-AqA 47 was found to be the most potent inhibitor of gastric ulcerogenic effects of indomethacin (Tables 1 and 2). However, PE-AqA and PE-AqA 47 did not antagonize the histamine-induced changes in gastric secretion volume, pH, acid-output and pepsin activity but attenuated potently the hypothermia and restraint-induced gastric effects (Tables 2 and 3). The gastric lesion formation and reduction in gastric mucus secretion by histamine, as well as hypothermic-restraint stress, were also blocked highly significantly ($P<0.001$) following pre-treatment with PE-AqA and PE-AqA 47 (Table 3). However, PE-AqA 47 exerted stronger effect than PE aqueous extract and PE-AqA, indicating that PE-AqA 47 fraction has probably extracted most

Table 3. Effects of *P. emblica* fruit extract and fractions on gastric ulcer index and mucus secretion in hypothermic-restrained rats.

Treatment)	Ulcer Index (mm)Mean±S.E.M.	Mucus Secretion (mg/h)Mean ± S.E.M.
Normal (Untreated Control)	2.62 ± 0.26	0.239 ± 0.008
Hypothermic-Restraint (Treated control)	26.99± 0.27**	0.182 ± 0.007**
Hypothermic-Restraint + Farmotidine (100 mg/kg p.o)	15.67± 0.51**	0.204 ± 0.006*
P. emblica aqueous extract (Eq. to 1.5 g/kg of Plant Drug p.o) + Hypothermic-Restraint	10.56± 0.46**	0.206 ± 0.005*
P. emblica aqueous-ethyl acetate fraction (Eq. to 1.5 g/kg of Plant Drug p.o) + Hypothermic-Restraint	9.64 ± 0.62**	0.209 ± 0.005*
P. emblica aqueous-ethyl acetate 47 (Eq. to 1.5 g/kg of Plant drug p.o) + Hypothermic-Restraint	4.32 ± 0.52**	0.245 ± 0.008*

Hypothermic-restrained: significant from untreated control (Normal)* $P<0.05$; **$P<0.001$
Test drugs: significant from treated control (Hypothermic-Restrained) *$P<0.05$; **$P<0.001$
Standard error of means (S.E.M) of six experiments

of the antiulcer active principles of the test plant. From the results obtained, it is conceivable that a dual antiulcer action has been exerted by the purified fraction, viz., inhibition of gastric aggressive factors i.e. acid and pepsin and strengthening of the mucosal barrier, as was also suggested for gefarnate by Barbara *et al*. [31].

In conclusion, the above reported results have validated the folkloric use of the plant drug tested for use in the therapy of gastric ulcer disease. In particular, the present studies have pointed out possible cytoprotective effects of the PE-AqA 47 fraction isolated from *Phyllanthus emblica* fruits. Nevertheless, detailed chemical studies, followed by pharmacological investigations and toxicity evaluations, are still required to isolate the active principles of the *P. emblica* fruit and to elucidate their precise mode of antiulcer action.

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A STUDY OF GENOTYPIC FREQUENCY OF VARIOUS BLOOD GROUP ALLELES

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Abstract: A random population sample from urban and rural areas of Bannu region, Northwest Frontier Province (NWFP) of Pakistan was screened for distribution of blood groups. The objective was to identify the genotypic frequency of blood groups in the region, in order to comprehend the allelic diversity. Blood grouping was carried out over a period of 16 months from January 2002 to April 2003, and encompassed 2581 subjects, in which 57.09% were male and 42.9% female. These were categorized according to ABO/Rh system. Allele frequency was computed according to Hardy-Weinberg law. The distribution of phenotypes in the total sample was 0.3623, 0.3103, 0.2507 and 0.0767 for groups B, A, O and AB, respectively, with 0.672 Rh-positive (R) and 0.328 Rh-negative (r). B group was dominant in both the genders, and AB was rare in both males as well as females. The distribution of the alleles in the total sample was 0.345, 0.378 and 0.277 for I^A, I^B and i, respectively. The studied population exhibited a predominance of group B, in the order of B>A>O>AB, as well as Rh-positive antigen for both male (90.26%) and female (87.98%) subjects within the population, with Rh-negative men and women being 9.74% and 12.02% respectively. Allele frequency recorded was in the order of I^B> I^A >i, and R>r,

Keywords: Alleles, gene frequency, blood groups, Rh factor, transfusion, Bannu, Pakistan

Introduction

Several blood group systems, based upon different antigens, have been proposed so far. Of these the ABO system described in 1900 by Karl Landsteiner has gained immense medical significance. In combination with the Rhesus system, the ABO system has become the recognized system for determining blood group compatibility for safe transfusion [1]. Blind mixing of blood can initiate an immune reaction. Only the blood samples, which share the same antigenic identity, do not initiate an immune response, and hence are deemed compatible.

Genes responsible for the ABO antigens appear to be located on the long arm of the autosomal chromosome number 9 [2]. A set of three possible alleles at an autosomal locus are responsible for the four blood types (O, A, B, AB). The gene symbols i or I^o, I^A and I^B, are often used to denote these alleles, where I stands for isoimmunoagglutinin. The superscript indicates the specific antigen. One of these (i=O), is recessive to the other two (I^A=A and I^B=B) and does not produce any antigen. Allele I^A produces antigen A and is codominant with the third allele I^B, which produces antigen B. These three alleles combine to yield six genotypes and four phenotypes. Two alleles, R and r, are responsible for the inheritance of rhesus blood groups, with R denoting Rh^{+ve}, and r being Rh^{-ve} blood group allele.

Gene frequency (the proportion of different alleles for a gene that are present in the population)

takes into consideration the numbers of various genotypes in the population, and the relative allele frequencies are determined by application of the Hardy-Weinberg Law [3]. Muller was the first investigator who examined the ABO antigens among Yoruba people of Nigeria, Africa. According to him the frequency of the alleles is: O=0.7271, A=0.1320 and B= 0.1409, and percentage of the blood groups being 24.43% A, 23.88% B, 2.75% AB and 48.94% O [4]. Thus in Africans O blood group is more prevalent, followed by A, B and AB. However, in Asians and Caucasians the prevalence of blood group B is the highest, the sequence being B>O> A>AB [5].

The present study was carried out to record the genotypic frequency of various alleles in the blood groups in a population of Bannu, Northwest Frontier Province (NWFP), Pakistan, with a view to generating data with multipurpose benefits to health planners.

Materials and Methods

Subjects

A total of 2581 subjects, comprising 1123 female and 1458 males, were screened for blood grouping. The subjects belonged to both rural and urban areas of Bannu, NWFP, Pakistan.

Collection of blood samples

A 2.0 ml sample of blood was drawn from the antecubital vein of each subject in a disposable syringe, and transferred immediately to a tube containing ethylenediamine tetraacetic acid (EDTA).

Determination of blood groups

Blood grouping (ABO and Rh) was done by the antigen-antibody agglutination test. The antisera used were obtained from Plasmatec (Kent, UK). Plasmatec ABO monoclonal reagents are *in vitro* culture supernatants of hybridized immunoglobulins secreting mouse cell-line. For determination of Rh factor, plasmatec anti D (1.0 g) Lo-Du and LO-Du2 monoclonal reagents, prepared from different antibody producing human B-lymphocyte cell lines, were used.

Computation of Allele Frequencies

Allele frequency of the antigens was computed by application of the Hardy-Weinberg Law [3], on the basis of the number of subjects with different blood groups.

Results

Table 1. Phenotypic frequencies of various blood groups (ABO and Rh) in the studied population.

Blood Group	Total Subjects			Male Subjects			Female Subjects		
	Complete	Rh^+	Rh^-	Complete	Rh^+	Rh^-	Complete	Rh^+	Rh^-
A	0.31030	0.3108	0.3069	0.3484	0.3496	0.3380	0.2609	0.2591	0.2741
B	0.3623	0.3637	0.3502	0.3608	0.3670	0.3028	0.3642	0.3593	0.4000
AB	0.0767	0.0686	0.1444	0.0480	0.0357	0.1620	0.1140	0.1124	0.1259
O	0.2507	0.2569	0.1985	0.2428	0.2477	0.1972	0.2609	0.2692	0.2000
Total	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 1 shows the phenotypic frequency of

ABO blood groups in the studied population, with gender distribution. The distribution of phenotypes in the total sample was 0.3623, 0.3103, 0.2507 and 0.0767 for groups B, A, O and AB, respectively, with 0.672 Rh positive and 0.328 Rh negative. B group was dominant in both the genders, and AB was rare in both males as well as females. Table 2 depicts the distribution of allele frequencies of ABO antigens in the studied population, in comparison with certain earlier studies. The distribution of the alleles in the total sample was 0.345, 0.378 and 0.277 for I^A, I^B and i, respectively. Table 3 compares the distribution of allele frequencies of Rh factor antigens in the Bannu population with earlier studies on different populations.

Table 2. Frequency of alleles among blood group (ABO) of different populations.

Population	Allele Frequency			Reference
	I^A	I^B	i	
Britain	0.496	0.331	0.173	[30]
India	0.318	0.452	0.230	[30]
Nigeria	0.356	0.479	0.165	[4]
Kenya	0.343	0.447	0.210	[5]
Hazara (Pakistan)	0.260	0.408	0.332	[9]
Swabi (Pakistan)	0.307	0.396	0.297	[10]
Peshawar (Pakistan)	0.327	0.408	0.265	[31]
Bahawalpur (Pakistan)	0.275	0.480	0.245	[12]
Bannu (Pakistan)	0.345	0.378	0.277	Present study

Table 3. Frequency of Rh antibody alleles among different populations.

Population Reference	Allele Frequency		
	R	r	
Lahore (Pakistan)	0.717	0.283	[32]
Nigeria	0.943	0.057	[4]
Kenya	0.803	0.197	[5]
Azad Jammu and Kashmir	0.848	0.152	[33]
Islamabad (Pakistan)	0.729	0.271	[34]
Peshawar (Pakistan) [29]		0.768	0.232
Bannu (Pakistan)	0.672	0.328	Present study

Discussion

A survey conducted by Wagner in South Western Germany designated the ABO allele frequency as O=0.0640, A=0.279, B=0.081 [6]. Estimate of the gene frequency for ABO system in Hungary has been reported as i= 0.5593, I^A= 0.2989 and I^B=0.1418 [7]. In Nairobi (Kenya), the percentage distribution of blood groups has been reported [5] as 0=47.4%, A=26.2%, B=22.0%, AB=4.4%. The relative gene frequencies were i=0.690, I^A=0.168 and I^B=0.142, 96.1% being Rh positive having gene frequency of 0.804 (R). Rh(D) negative were only 3.9% with the gene frequency (r) being 0.0196 [5]. In the United States, 85% of the Caucasians and 92% Negroids have been described as Rh^{+ve}, while the Japanese, Chinese and pure American Indians have been found to be 99% Rh^{+ve} [8]. Prevalence of $Rh^{+ve,}$ has been reported as 100 % in China, 84% in Europe and 94.6% in W. America [9].

India and Pakistan both have a higher frequency of group B as compared to Europe and U.S.A. [1,9,10]. In Pakistan, work has been done on blood

group distribution in all the provinces. In Wah cantonment (Punjab), the gene frequency distribution is I^A= 0.203, I^B= 0.255, i= 0.542, R= 0.730 and r= 0.271 [11]. In Bahawalpur City, the percentage of blood groups O is 37%, A 21%, B 36% and AB 6% [12], while in Bannu (NWFP) it is 31.03%, 36.23%, 7.67% and 25.07%, for A, B, AB and O blood groups, respectively [13].

In the study under discussion, the relative frequency of the various blood groups does not seem to deviate from those which have been recorded for studies on various other segments of the Pakistani population (Table 2). However, comparison with the data from the British and African populations (Table 2) reveals that there is an equal dominance of group B and O in the Indo-Pak subcontinent, in contrast to dominance of only O group in the British and African populations. The least reported group, in all the populations, has been AB.

It has been reported [14-16], that in the populations of the United States, Asia, Syrian Arabs and Palestinian region, group O is dominant, with AB being the rarest. Racial (genetic) and environmental factors have been reported to influence the frequency of various blood groups in studies carried out in various societies, including Bangladesh and Latin America [17-20]. The genetic and environmental factors responsible for varying frequency of the blood groups among the Pakistani populations need to be probed further.

In terms of presence of Rh antibodies, the data from several studies on Pakistani as well as certain African populations are compared in Table 3, along with the allele frequency of R and r. The present study shows a comparatively higher percentage of Rh-negative cases, though it is still very low and follows the global trend of being significantly rarer than Rh-positive individuals. The findings of the present study are inconsistent with the results obtained in an earlier study carried out in Wah Cantt (Pakistan), where the allele frequency of Rh-positive (R) has been found to exceed the Rh-negative (r) cases (0.730 and 0.271, respectively) [11].

An association with the blood groups with several diseases, especially cardiovascular diseases, which has been reported over the years [21-29], would make the data generated in the present study useful for health planners. The study provides in depth information of the relative distribution of various alleles in the population and promises help in planning for future health challenges in the region.

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MEN'S ATTITUDES AND THEIR PARTICIPATION IN FAMILY PLANNING PROGRAM: A MICRO LEVEL STUDY IN BANGLADESH

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Abstract: The analytical findings revealed that knowledge of men in family planning (FP) methods is universal; their attitude on FP program is positive but contraceptive use status is not satisfactory. About 17 percent of the married men are the users of FP methods. Contraceptive prevalence rate of the couples is 71 percent. Oral contraception (Pill) is the most used method, which is about 37 percent followed by condom 15.9 percent. Mean age at marriage of the respondents is 22 years and for the spouses is 16 years. There is a positive correlation between husband and wife's education. Among the respondents, 63 percent have school education and the corresponding figure for spouses is 52 percent. Service is the main occupation of the respondents (48 percent). Monthly average income of the respondents is approximately $40. Majority (78 percent) contraceptive users of male do not support female methods. Regarding reasons for using male methods, the majority users (58 percent) mentioned that they have fewer side effects. Our findings revealed that desire for additional children, education of men, age of men and joint decisions on family matters by couples are the most significant determinants of contraceptive use. This analysis further indicates that the likelihood of contraceptive use among the men desiring no additional children is 1.88 times higher than those who aspire children. Men who have at least school level education have 1.81 times higher contraceptive-using tendency than the men who have no education. Husbands who share family decisions with wives have 50 percent higher contraceptive using status than self-decision makers.

***Keywords*:** Men's attitudes, family planning, education, occupation, age at marriage, decision making status, mass media.

Introduction

Men's involvement in family planning, either as users of male methods or as supportive partners of users, has largely been ignored by family planning programmers and service providers. Because of women's unique role in reproduction, highly modern contraceptives have been developed over the past few decades for them. However, demographers and population specialists have also turned their attention to men's reproductive behavior [1,2]. This is because, in many third world countries, men play an important role in household decision making processes, including desirable family size and use of family planning (FP) methods.

Many women do not use FP method because their husbands oppose it. Men's reason for opposing family planning varies by socio-economic characteristics. Some want more children while the others oppose contraception even if they do not want to have more children; they worry that their wives might be unfaithful if protected from pregnancy. Some men are jealous that male physicians would examine their wives. Still others want to control their wives' behavior due to religious objections or fear of side effects of FP method. Husband's attitudes may affect not only wives' intention to use contraception but also the choice of a method and how long it is being used. In Bangladesh, men often give their wives permission to practice contraception in a noncommittal way, without actually making a decision themselves; if anything goes wrong, they can blame their wives. Men have authority, but often they are reluctant to take responsibility [3].

Men's attitude towards FP methods and ideal family size are of enormous importance for fertility. Use of contraception by a woman and its continuation is extremely difficult without positive attitude of her husband. Male involvement refers to the ways in which men relate to reproductive health problems, reproductive rights and reproductive behavior [4]. Male involvement in family planning has two major aspects: (i) the way men accept and support their partner's need, choices and rights in fertility regulation, and (ii) men's contraceptive use and sexual behavior [5]. It has been found in several studies around the world that husbands' active disapproval led women give up use of contraceptives [6]. According to data in the 1989 Kenya demographic and Health Survey, knowledge and approval of family planning, education of husband and wife, husband-wife communication, desire for more children and ideal family size are all significantly associated with current use of family planning methods. Husband-wife communication, particularly the wife's perception of her husband's approval of family planning is highly associated with contraceptive use [7].

There is no general and clear correlation between poverty and fertility. Rodgers [8] states that there is no evidence from Bangladesh, India, Nepal and Pakistan that the poor have relatively high fertility. In fact, there is a hint that their fertility may be lower than in at least the middle-income groups. Such a correlation has been demonstrated for Bangladesh in the 1960s and again in the late 1980s [9]. The message in mass media provides important information on the choice of methods, cultural values of contraception, how to use them and their availability and accessibility. It is revealed from the Bangladesh Demographic and Health Survey (DHS) of 1993-94 that the media messages about any FP methods are acceptable to 77 percent of the married couples in their child-bearing age. However, there is a considerable gap between the acceptance of media message and the actual performance of contraceptive use [10]. Many studies have been conducted on the dynamics of family planning adoption but demographic research has historically focused on the determinants of contraceptive use by women. Women were typically the respondents in the Knowledge, Attitude and Practice Surveys, the World Fertility Surveys, the Contraceptive Prevalence Surveys, and the first round of the Demographic and Health Surveys (DHS). Very recently, attention has been given to studying the determinants of contraceptive use among men. DHS surveys that included both women and men have been conducted in more than 20 developing countries. These data reflect gender differences in reproductive behavior and fertility preferences and the husband's influence in decision-making regarding family size and family planning adoption [11].

Efforts to promote family planning in developing countries have often been criticized for their exclusion of men. The consequences of the female-only approach have been that some men view family planning with suspicion, consider it as being aimed at undermining their authority in the family. For instance, men in Nigeria typically believe that contraception makes it easy for their wives to engage in extramarital sexual relationships [12]. While men's attitudes toward family planning are generally positive, some studies show that men believe that they should be in control of whether and when a couple uses contraceptives [13,14]. Recent research has also provided evidence that supportive husbands increase the likelihood of contraceptive use and once a method is begun it is continued. In this study attempts have been made to assess men's role, belief, practice and their participation in family planning programs.

Materials and Methods

The sample size for the study was only 600 men

(fourth-class employees) and other lower income groups who are residing within and around the university campus. In addition to fourth-class employees, rickshaw pullers, petty businessmen and day laborers were also considered in the sample. The data used for the study are not representative as it is a micro level case study. A total of 600 married men were interviewed using a structured questionnaire to meet the objectives of the study. To know the socio-cultural, demographic and reproductive behavior of the respondents, univariate analysis was carried out. Logistic regression analysis was carried out to determine the influential factors of contraceptive use. This regression model was used to describe the relationship between an outcome (dependent or response variable) and a set of independent (predictor or explanatory) variables. In our study, the response variable was current contraceptive use status of men. Out of the 600 men, some responded "no = 0" and others "yes = 1". When the explanatory variables have two categories and if one category is represented by 0 (Zero) then the logit model equivalent to log linear model and logit model for logistic regression model will be the same results [19]. Let Y_i denote the dependent variable for i-th observation and $Y_i = 1$ if the i-th individual uses contraceptive and $Y_i = 0$ if i-th individual does not use. Consider a collection of multiple (p) independent variables which will be denoted by the vector $X' = (x_1, x_2, x_3, ... x_p)$ and the vector of the respective coefficients of X is $\beta' = (\beta_1, \beta_2, \beta_3, ... \beta_p)$. These variables are either qualitative or quantitative. Let the conditional probability be denoted by $\Pr(Y_i=1/X) = \pi(Y)$. Then the logit of the multiple logistic regression model is given by the equation

$$g(X) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_p X_p \quad (1)$$

In which case,

$$\pi(Y) = \frac{e^{g(X)}}{1+e^{g(X)}} \quad (2)$$

and

$$\Pr(Y_i = 0/X) = 1-\pi(Y) = \frac{1}{1+e^{g(X)}} \quad (3)$$

Equations (2) and (3) look complicated. However, the logarithm of the ratio of p (Y) to 1-p (Y) turns out to be a simple linear function of X, that is,

$$Logit\pi(Y) = \log_e \frac{\pi(Y)}{1-\pi(Y)} = \beta' X \quad (4)$$

The equation expresses the log odds of occurrence on an event as a linear function of the independent variables. The logit is thus the logarithm of the odds of success, that is, the logarithm of the ratio of the probability of success to the probability of failure. It is also called the logit transformation of p (Y) and equation (4) is a linear logistic model. It has several nice properties, p (Y) is bounded only between 0 and 1. If p (Y)<0.5, logit p (Y) is negative; while if p (Y)>0.5, the logit p (Y) is positive. In our study, contraceptive use status was considered as dependent variable. If the respondent was a current user then it took a value of unity and if the respondent was not a current user, it took a value of zero. The independent variables were considered as x1= age of the respondents, x2= education of the respondents, x3= income, x4= age of youngest child, x5= want more children, x6= decision making on household matters, x7= occupation of the respondents and x8= watch FP program on Radio/TV.

Results and Discussion

In order to understand the male knowledge, attitudes and contraceptive use behavior, it is important to know the economic, demographic and reproductive behavior of the respondents. Table 1 shows that average age of respondents was 33 years. The information supports that 44 percent of the respondents reported that their age at marriage was between 20-25 years and the average age at marriage of men was about 22 years. Roughly half of the wives were married at an age of 12-16 years. Early marriage for females is customary in Bangladesh. In rural areas, most girls marry before the age of 12 years [15,16]. The average age at marriage of the respondent's spouse was about 16 years, although, in 1976, the minimum legal age at marriage in Bangladesh was fixed at 21 and 18 years for males and females respectively by the government. Education influences contraceptive behavior. It facilitates a shift toward a conjugally oriented relationship in which the husband and wife are more likely to take into account the interests of the other sex and of the conjugal unit as a whole [17]. In our study educational attainment of the respondents is a somewhat better than generally

found in most surveys. This is because a majority of the respondents were university employees. Sixty three percent of the respondents ever attended school. The corresponding result of ever-attended school is 60 percent in the study of 1999-2000 BDHS survey report (18). The average years of schooling in our study were 5 years. There was a positive correlation between education of husband and wife. About 52 percent of the respondent's wives ever-attended school and the average years of schooling of wives were 4 years. Occupation of the respondents is also an important indicator of the socio-economic status. Two major occupations namely, service (48 percent) and rickshaw pulling (34 percent) were frequently reported by the respondents. The major occupation of wives was housewife (96 percent). Service was the main source of income of the respondents. Income distribution shows that the average monthly income of respondents was Tk. 2501 (approximately $40).

Table 1. Percent distribution of the respondents by background characteristics**.**

Characteristics	Frequency	Percentage
Age		
Less than 24 years	66	11
24-30	180	32
30-36	170	28
36-42	112	19
42 and above	62	10
Age at first marriage		
Less than 15 years	18	03
15-20	206	34
20-25	266	44
25 and above	110	18
Level of respondent's education		
No schooling	222	37
1-5 years schooling	166	28
6-10 years schooling	182	30
11+ years schooling	30	5
Wife's level of education		
No schooling	288	48
1-5 years schooling	180	30
6-10 years schooling	128	21
11 years and above schooling	4	01
Respondent's Occupation		
Rickshaw pullers	206	34.4
Service	190	48.3
Business	50	8.3
Day laborer	54	9.0
Wife's occupation		
Housewife	578	96.3
Service	16	0.7
Poultry	4	0.3
Maid servant	2	2.7
Monthly income		
Less than Tk. 1600	62	10.3
Tk. 1601-Tk. 2400	216	36.0
Tk. 2401-Tk. 3200	242	403
Tk. 3201 and above	80	13.3

Men can participate in family planning in two ways: by supporting their partner's decisions to use family planning methods and/or by practicing a male method of family planning themselves (condom, vasectomy, withdrawal or periodic abstinence). Man's support affects the choice, adoption, continuation and correct use of female methods. The level of general awareness about family planning is nearly universal today in Bangladesh. Virtually all couples know at least one modern family planning method. Results from 1999-2000 Bangladesh Demography and Health Survey (BDHS) show that among the current married couples knowledge of at least one contraceptive method is almost universal [18]. Many surveys in Asia show that a high percentage of couples have knowledge of FP methods and a favorable attitude towards their use. Yet only some of them adopt family planning. The information found in our study shows that knowledge of men on FP methods is universal and it appears that condom and pill are the most known methods followed by tubectomy and injection. The information on current use of contraceptives (Table 2) suggests that about 71 percent couples were currently using contraception but the present contraceptive using rate of Bangladesh is almost 54 percent [18]. Contraceptive Prevalence Rate (CPR) may be much higher as the respondents belong to an educated community and they can realize the benefit of small family size norms. Among the methods in use, 44.7 percent were female methods and 16.8 percent were male methods. Traditional methods used by both husband and wife were 9.6 percent. The most popular methods were pill (used by 37.3 percent) followed by condom (used by 15.9 percent). The corresponding figures according to1999-2000 BDHS data [18] were 23 and 4 percent, respectively.

Table 2. Percent distribution of respondents by current use of family planning methods.

FP Methods	Number	Percent
Pill	224	37.3
Condom	98	15.9
Tubectomy	28	4.7
Injection	16	2.7
Vasectomy	6	0.9
Traditional methods	54	9.6

In our society, men play main role over women in deciding whether they use any family planning method. They have important say in decisions such as desired family size and the use of family planning methods. Therefore, an assessment of men's attitudes towards family planning would provide indication about their role in the choice of family planning methods. Among the users, a majority of husbands did not support female methods (78 percent) and preferred male methods using arguments shown in Table 3.

Table 3. Distribution of respondents by reasons for using male methods.

Reasons	Number	Percent
Less side effects	56	58.2
Wife likes it	4	4.1
Easily available	20	20.4
Easy to use	4	4.1
Delay to next pregnancy	6	6.1
Low price	4	4.1
Lower risk	4	4.1
Both like it	8	8.2

Note: Percentage may not add to 100 because of multiple responses.

To investigate the factors that have influence on the men's participation in the use of family planning methods, a number of independent variables were considered and logistic regression analysis was used. The independent variables used were:

x_1 = age of respondents:	If respondent is less than 35 years =0If respondent is aged 35 years and above =1
x_2 = Education status of respondents:	If respondent has no education =0If respondent has education =1
x_3 = Income of respondents:	If income is less than Tk. 2000 =0If income is above Tk. 2000 =1
x_4 = Age of youngest child:	Age is less or equal two years = 0Age is more than two years = 1
x_5 = Want more children:	No = 0 Yes = 1
x_6 =Decision making :	Self decision by husband/ wife= 0Husband and wife take decision=1
x_7 =Occupation of respondents:	If the respondent is other than service=0If the respondent is in service =1
x_8= respondents regularly watch the FP program in TV/Radio:	No =0 Yes=1

The dependent variable is the current contraceptive use status of men**.**

The results of the logistic regression analysis are shown in Table 4. The most significant variables that influence men's current uses of contraception are desire for additional children followed by educational status, age of the men, decision on household matters and role of mass media. Among the men who are not interested in having another baby, contraception rate is significantly higher (88 percent) compared to its rate for those who want more children. Education is the second important determinant of contraceptive use. In case of men who have formal education, the contraception rate is 81 percent higher than the others. Age is an important determinant of contraceptive use by male respondents. The higher their age of male, the higher was the likelihood of contraceptive use. In this study, contraceptive use status was higher (62 percent) for men whose ages exceeded 35 years than the men of other ages. The male partners play an important role in decision-making regarding contraceptive use and the timing and number of couple's births. Joint decision of husband and wife is another important determinant of FP program. In case of couples who take decisions on family matters jointly, the contraceptive using status was significantly higher (50 percent) compared to couples where only men take decisions. Similarly, education, service status and mass media turned out to be positively associated with use of contraception.

Table 4. Logistic regression analysis with current use of contraception being the dependent variable.

Variable	Coefficient	Sig.	Odds ratio
x_1: **Age**			
<35 years **(RC)**	-	-	1.00
≥35 years	.4841*	.0280	1.62
x_2: **Education**			
No **(RC)**	-	-	1.00
Yes	.5936*	.0037	1.81
x_3: **Monthly income**			
<2000TK. **(RC)**	-	-	1.00
>2000 TK.	.3129***	.1031	1.36
x_4: **Age of the youngest child**			
≤2 years**(RC)**	-	-	1.00
>2 years	.2392	.2161	1.27
x_5: **Whether want additional child**			
Yes (RC)	-	-	1.00
No	.6336*	.0007	1.88
x_6: **Decision on household maters**			
Self decision (RC)	-	-	1.00
Joint decision by couples	.4292	06608	1.50
x_7: **Occupation of the respondents**			
Other (RC)	-	-	1.00
Services	.3666*	.0692	1.44
x_8: **Watch radio/TV**			
No (RC)			1.00
Yes	.4012*	.0712	1.49
Constant	1.0709	.0000	
- 2 log likelihood	χ^2 = 216.11, P<.001		
Significance	* P<. 001, ** P<. 005, *** P<01.		

Conclusion

In this study men's attitude and participation in family planning program was explored using only 600 respondents. Although the sample size (600) may not be enough to draw a decisive conclusion, it is expected that the study would provide some indication of the role and responsibility of men in family planning programs. Since the family planning program of Bangladesh is mainly female oriented, the information generated would help the policy makers in adopting effective strategies for involvement of men in the future family planning programs. Mass media plays a significant role in increasing the male participation rate in family planning. Operations research conducted in Bangladesh indicates that both men and women have responded favorably to programs that have focused on family planning and reproductive health information and services [2]. Logistic regression analysis suggests that the significant factors that may motivate men to participate and use contraceptives include age, educational

attainment, desire for additional children, decision about household matters and occupation of the respondents. The analysis also suggests that access to mass media activities for promoting family planning in Bangladesh should continue to focus on the importance of dialogue between men and women. Since the current family planning program of Bangladesh is targeted towards women, there is little information about men's roles and responsibilities. Family planning research, policy and programs must continue to expand men's participation and focus on men's attitude towards contraception.

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PLANT CHARACTERS IN RELATION TO EARLINESS IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract: Fifteen phenotypically diversified genotypes were evaluated for seven characters in relation to earliness. According to analysis of variance, the genotypes differed significantly for all the characters studied. They varied from 6.5 to 9.3 in sympodial node numbers; 19.5 to 43.1 cm in sympodial length; 3.5 to 6.1 cm in internode length; 80.6 to 149.4 cm in plant height; 34.7 to 79.2 cm in leaf size; 2.9 to 4.4 gm in boll weight; 34.3 to 93.4 % in earliness (bolls opened at 125 days after planting) and 59.5 to 126.2 gm in seed cotton yield. It is also observed that none of the genotypes was simultaneously favored with all the earliness characters; however two varieties, CRIS-134 and CRIS-220 were generally favored with greater number of earliness characters against the other genotypes. Therefore, these can be regarded as short season cottons. These short season genotypes produced sympodial branches at below 7.3 nodes; short sympodial branches under 23.8 cm; short internodes upto 4.3 cm; shorter plants of 96.9 cm; smaller leaves upto 37.9 cm; moderate bolls weighing upto 3.4 gm; percent of open bolls upto 89.9 at 125 days after planting. It is assumed that short season cotton in Pakistani conditions could be considered as 125 to 150 day crop. Thus if these two short season cottons were picked at 150 days, they would have opened more than 95% of their bolls. Both the short season cottons though gave significantly lower yields than many other varieties in the test, yet CRIS-220 still gave an acceptable yield because some of the long season cottons such as BH-118 yielded even less (62.3 gm) than both the short season cottons. Thus yield may be more related to genetic potential of a variety rather than earliness. A third variety CRIS-226 not favored with as many earliness criteria as CRIS-134 or CRIS-220, showed moderate sympodial length (26.4 cm) and boll weight (3.4 gm) While this variety opened maximum percent of bolls (93.4 %), it still gave fairly good yield of 116.4 gm. Our results therefore generally reveal that all the morphological traits studied have fairly good relationship with earliness and hence could reliably be used as selection criteria for breeding short season cottons.

Keywords: Sympodial branches, short season cotton, boll weight, plant height, yield

Introduction

Short duration cottons are desirable in that they require less fertilizer, irrigation and labor. They are also exposed for shorter period to the environment as compared to long duration cottons. Hence short season cottons provide as escape from many harmful insects, especially from late season cotton bollworms. These are also of great importance in the country like Pakistan where sequence of other crops succeeds the cotton. In Pakistan, wheat is sown right after cotton. Thus long duration cotton becomes a serious problem to the growers because it encroaches the prescribed sowing period of wheat. Short season cotton also fits better in double and triple cropping system, maximizing the cropping intensity and increases crop production.

In Pakistan, efforts are being made for developing short season cotton but the selection criteria for such type of cottons are yet to be established. Uzbek and Indian breeders have already made headways in developing short duration cotton varieties, which are early maturing and good yielders

with desirable fibre traits simultaneously. A number of selection criteria, however, have been proposed which determine earliness. These are: reduction in monopodia, profuse flowering, high early boll setting, fewer leaves, short internodes, semi-determinate types, lower sympodial node number, short sympodial branches, cluster fruiting types, moderate boll size, sub-okra and dwarf stature [1]. Godoy [2] worked on several early lines and one full-season cultivar to obtain information on earliness parameters. It was noted that number of nodes to first fruiting branch, plant height, date of first flower and date of first open bolls were efficient criteria to identify early cotton varieties. Solis *et al*. [3] carried out field trials on 18 cotton genotypes having substantial variability in phonological and yield attributes. The genotypes were categorized as early, intermediate and late maturing. They concluded that days to first flower provided a reliable criterion of earliness. Ray and Richmond [4] observed that node number of first fruiting branch is a good estimator of earliness in cotton.

In India, Kairon and Singh [1] considered some above mentioned morphological characters of short season cotton and succeeded in reducing growing period by 80-95 days in *hirsutum*, 20-25 days in *arboreum*, 55-60 days in *harbaceum*, 45 days in *barbadense* and 50-55 days in commercial cotton hybrids. In china, short duration varieties like Zhong Mian Suoto with 115-120 days duration and Heishan Mian-1 of 117-122 days duration are also under cultivation. Del Cerro and certain storm proof cottons of USA are also reported to be early, besides some super okra types having reduced stature and earliness in maturity.

Because of specific climatic conditions, high cropping intensity, shortage of irrigation water, high input costs of cotton in Pakistan, cotton breeders are very enthusiastic to develop early or medium duration cotton varieties. The present study, therefore, was carried out to evaluate the existing commercial as well as promising strains for their earliness in maturity and to categorize them accordingly so that the growers may be advised regarding their cropping pattern.

Materials and Methods

Fifteen cotton genotypes including commercial genotypes (VH-137, FH-901, CRIS-134, FH-900, BH-118 and CIM-473) as well as advance strains (CRIS-168, CRIS-120, Cyto-51, CRIS-212, CRIS-226, CRIS-121, CRIS-468, CRIS-220 and CRIS-467) with distinguishing morphological traits were evaluated for their plant architecture in relation to short duration cottons. The characters studied were: first sympodial node number on main stem, sympodial branch length (cm), internode length (cm), leaf size (cm), plant height (cm), boll weight (cm), earliness (percent of bolls opened at 125 days after planting) and seed cotton yield (g). The experiment was laid-out in a Randomized Complete Block Design with four replications arranged in a plot size of 1372.5 x 381.25 cm. Ten plants from each replication and genotype, totaling 40 plants, were randomly tagged for recording the observations. All inputs such as fertilizer, irrigation and insecticides were given as and when needed. The analysis of variance according to Steel and Torrie [5] was carried out so as to work out the difference among the genotypes for various morphological, earliness and yield characters. The least significant differences at 5% probability level were used to separate the means of genotypes for different characters.

Results and Discussion

Short season cotton has become an important objective of cotton breeders in Pakistan for several reasons. It fits better in cotton-wheat

rotation, is desirable in double and triple cropping system, provides an escape from many harmful insect-pests and requires less irrigation, fertilizer, insecticides and labor.

Fifteen genotypes with morphologically distinguishing characters were evaluated for establishing any relationship of these characters with early duration cotton. There existed significant differences among genotypes for various developmental, earliness and yield characters (Tables1, 2). The averages of morphological characters that were considered to be related to earliness or short season cotton are given in Table 3. The genotypes differed significantly in producing first sympodial node that varied from 6.5 to 9.3 numbers on main stem. It is generally assumed that the lower the sympodial node on the main stem, the earlier is the variety. Ahmed and Malik [6] estimated that one node decrease in sympodial branch matures the crop by approximately 4 to 7 days. Several other workers [1,3,7,8,] have also reported strong relationship between earliness and lower sympodial node number on the main stem. The US cotton breeders consider the short duration cotton as the one, which bears 1st sympodial branch at 4th to 5th nodes, while long duration cotton sets fruiting branches at 8th to 9th nodes. Out of the 15 genotypes evaluated, 6 of them (CRIS-120, CRIS-212, CRIS-121, CRIS-467 and CIM-473) produced 1st fruiting branch in the range of 6.5 to 7.0 nodes, which can be regarded as short or medium season cottons.

The genotypes also differed significantly in sympodial branch length, which varied from 19.5 to 43.1 cm. However, CRIS-226, BH-118, CRIS-134, CRIS-220 and FH-900 were among the top five genotypes which gave relatively short sympodial branches ranging from 19.5 to 26.4 cm. Breeders in Uzbekistan have succeeded in breeding early maturing cotton by developing varieties with shorter

Table 1. Mean squares from analysis of variance of varieties evaluated for plant architecture (sympodial no., sympodial length, internode length, plant height) in relation to short season cotton.

Source of variation	Degrees of freedom	Mean Squares			
		Ist sympodial node number	Sympodial length (cm)	Internode length (cm)	Plant height (cm)
Replication	3	0.02	15.71	0.76	40.56
Genotypes	14	4.04**	210.79**	2.01**	1255.02**
Error	42	0.57	14.64	0.37	87.06

* Significant at 1% probability level

Table 2. Mean squares from analysis of variance of varieties evaluated for plant architecture (leaf area, boll weight, earliness, yield) in relation to short season.

Source of variation	Degrees of freedom	Mean Squares			
		Leaf area (cm)	Boll weight (g)	Earliness %	Yield/ plant (g)
Replication	3	65.92	0.02	25.52	16.94
Genotypes	14	742.91**	0.95**	1137.59**	3540.81**
Error	42	36.88	0.02	8.47	14.66

** Significant at 1% probability level

Table 3. Mean performance of varieties/strains evaluated for plant architecture in relation to short duration cottons in *Gossypium hirsutum* L.

Gerotypes/ Strains	Ist Symp. node no.	Symp. length (cm)	Internode length (cm)	Plant height (cm)	Leaf area (cm)	Boll weight (g)	Earliness %	Yield/pl. (g)
CRIS-168	8.0	35.7	4.8	101.3	79.2	3.3	74.6	91.7
CRIS-120	7.0	29.4	4.8	98.8	64.4	3.7	65.7	109.5
Cyto-51	9.0	38.3	6.1	111.9	73.5	3.3	56.1	71.5
CRIS-212	6.8	34.0	4.8	108.1	57.0	4.4	76.2	174.0
CRIS-226	7.8	26.4	5.2	115.0	54.2	3.4	93.4	116.4
CRIS-121	6.8	27.4	4.5	93.1	54.9	3.7	58.2	106.6
VH-137	9.3	34.9	5.1	139.4	71.2	4.4	52.5	126.2
FH-901	7.5	31.9	3.9	121.9	58.3	4.4	75.8	96.3
CRIS-468	9.3	37.9	5.5	149.4	71.4	2.9	65.0	102.4
BH-118	9.3	25.3	3.5	103.1	51.1	3.4	34.3	62.3
CRIS-134	7.3	19.5	4.3	80.6	34.7	3.0	78.1	65.5
CRIS-220	6.5	23.8	3.7	96.9	37.9	3.4	89.9	91.1
FH-900	7.3	21.6	4.5	101.9	38.6	3.7	66.3	59.6
CRIS-467	7.0	41.3	5.4	118.8	49.7	3.1	79.8	91.6
CIM-473	7.0	43.1	4.9	120.0	48.3	3.9	61.4	123.2
Average	**7.7**	**31.3**	**4.7**	**110.7**	**56.3**	**3.6**	**67.9**	**99.2**
LSD (5%)	**1.08**	**5.5**	**0.87**	**13.3**	**8.7**	**0.21**	**4.2**	**5.5**

sympodial branches. Kairon and Singh [1] also rated the cotton varieties with short sympodial branches as early maturing and short duration cottons. The internode length is also considered as one of the specified criteria for short season cotton. The genotypes evaluated were significantly different in this parameter also. The internode length of the genotypes varied from 3.5 to 6.1 cm. Shorter internodes in the range of 3.5 to 4.5 cm were recorded by the varieties CRIS-121, FH-901, BH-118, CRIS-134, CRIS-220 and FH-900. Indian breeders are using short internode germplasm as a reliable criterion for breeding short season cotton [1].

Plant stature has also been associated with early or late season cottons. The Indian breeders have established that plant stature is correlated with earliness [1]. They further defined that short stature cotton is either determinate or semi-determinate in growth and these growth habits are also related to short season cotton. The genotypes evaluated differed for plant height, which varied from 80.6 to 149.4 cm. In Pakistani conditions, the short season cotton may be the one, which measures below 100cm plant height. Among the 15 genotypes evaluated, four of them (CRIS-120, CRIS-121, CRIS-134 and CRIS-220) measured 80.6 to 98.8 cm plant height.

Cotton plant is quite variable in leaf size, which can be grouped as small, medium and broad leaf types. They are even different in leaf shapes like normal to okra types. It has been observed

that the smaller leaf cottons mature earlier than broader leaf cottons and okra types than the normal leaf types. We have measured leaf area (leaf length x leaf width) to distinguish between smaller and broader leaf genotypes and determined their relationship with earliness. The genotypes had the leaf size in the range of 34.7 to 79.2 cm. In our studies, the leaf area under 50.0 cm was considered as medium leaf size. The varieties which measured either smaller or medium (under 50.0 cm) were CRIS-134, CRIS-220, FH-900, CRIS-467 and CIM-473.

It is well established that boll size has strong negative association with earliness. Therefore, Pakistani breeders have always made compromise to select for moderate boll size with desirable level of crop maturity and yield. Indian breeders have noted that medium boll size (3.5 to 4.0 g) is a reliable criterion for developing short season cotton varieties with still good yields. The genotypes tested produced bolls weighing 2.9 to 4.4 g as moderate bolls. However, we have considered varieties weighing bolls in the range of 3.0 to 3.5 g as moderate bolls. Among the 15 genotypes evaluated, the eight genotypes that gave bolls of moderate size were: CRIS-168, Cyto-51, CRIS-226, CRIS-468, BH-118, CRIS-134, CRIS-220 and CRIS-467. Tunio *et al*. [9] also noted that early maturing varieties produced relatively smaller bolls but still gave good yields against late maturing cottons.

Percent of boll setting and opening are major criteria for Pakistani breeders to select cotton varieties for earliness in maturity. Indian breeders however have made three classifications of maturity based on 90% of the bolls harvested. According to their classification, short duration cotton matures in 125-145 days, medium duration in 145-165 days and long duration in 170-190 days [1]. Our cotton crop is normally harvested in 150-165 days after planting with about 80% boll opening. However, these cottons cause some delay in wheat planting if the crop is left for 2nd or 3rd pickings. It means, though we are growing medium duration cotton, yet there is demand for short duration cotton for many reasons, especially for wheat plantation. Our varieties differed significantly for earliness and their percent of open bolls after 125 days of planting which varied from 34.3 to 93.4. The top four early maturing varieties CRIS-226, CRIS-220, CRIS-467 and CRIS-134, however, opened bolls in the range of 78.1 to 93.4%. Though, the boll opening observations were taken after 125 days and if pickings were extended for 20 more days (i.e. 125-145 days) these varieties would have opened above 90% of the bolls. Thus, these could be considered as short duration cottons.

It is generally believed that characters related to earliness impose adverse effects on the yield. For example, taller plants could produce more sympodial branches, thus greater number of bolls against short stature plants. Also, if number of bolls for any variety is kept constant, the bigger bolls of long duration cotton could yield more than the short season cotton. Longer sympodial branches of long duration cotton could produce more fruiting positions and thus could set greater number of bolls against shorter sympodial varieties. Shorter internodes of early maturing cotton may reduce the average plant height and thus could decrease the yield as explained earlier. Boll weight is also an important yield component and if number of bolls is kept fixed, the bigger bolls of long duration cottons could give more yield than the smaller bolls of short duration cottons. Keeping in view these facts, a compromise level needs to be determined where the shorter season cottons may be developed without causing significant losses to cotton yields. Our results in Table 3 suggest that CRIS-226, which is the earliest of all the varieties, still ranked third good yielder and other varieties CRIS-220 and CRIS-467 also gave better yields. Uzbekistan breeders have succeeded in developing quite a number of world's earliest maturing, high yielding, quality cultivars such as C-6037, Termez-14, Termez-16, Termez-24 and Karshin-8 [10].

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FATTY ACID COMPOSITION OF LIPID CLASSES OF *BASELLA RUBRA* LINN

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Abstract: The seed oil of *Basella rubra* Linn was classified by thin layer chromatography into neutral lipids (96.1%) and polar lipids (3.9%). The neutral lipids identified were hydrocarbons (2.7%), sterol esters (3.9%), triacylglycerols (59.6%), free fatty acids (4.5%), I, 3-diacylglycerols (8.7%), 1,2-diacylglycerols (6.8%), sterols (2.5%) and mono acylglycerols (7.4%). The polar lipids were phosphatidylethanolamines (1.3%), phosphatidylcholines (1.4%) and lysophosphatidylethanolamines (1.2%). The fatty acid range was from $C_{16:0}$ – $C_{18:3}$ showing higher percentage of unsaturated acids. The major fatty acids were palimtic, stearic, oleic and linoleic acids.

***Keywords*:** Triacylglycerols, fatty acids, thin layer chromatography, gas chromatography.

Introduction

Basella rubra Linn (Indian spinach) commonly known as goosefoot belongs to the beet family, Chenopodiaceae. This family consists of 102 genera and 1400 species found throughout the tropical lands of Asia and Africa, especially along seashore and in saline areas [1]. *Basella rubra* is a climbing ornamental herb with broad ovate or cordate leaves and white or red sessile flowers. It grows wild and is cultivated in different areas of Sindh and Punjab. The herb is acrid and sweetish, and has healing soporific, narcotic, aphrodisiac, fattening and laxative effects. It can serve for coloring jellies and other foodstuff [2]. The present investigation deals with its lipid constituents. Although studies on *Basella rubra* have been carried out previously [3-9], the present work on its seeds is a thorough and systematic extension of the earlier studies. The PCSIR Laboratories have carried out similar studies on *Crotalaria juncea, Seasamum indicum, Mimusops elengi* L, *Carum capticum, Pistacia khinjuk* and *Nicotiana tobacum* L of local origin [10-15] respectively under its annual research and development programmes.

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Materials and Methods

Extraction of Lipids

The total lipids from 15 g of powdered seeds were extracted with 300 ml chloroform and methanol (2:1, v/v) mixture at room temperature by shaking on a magnetic stirrer for half an hour. After filtration, the residual material was treated three times with 100ml of chloroform methanol mixture as above. All extracts were combined and washed with 100ml chloroform: methanol:aqueous sodium chloride (0.9%) (3:48:47, v/v) in a separating funnel. After removal of non-lipid impurities, the solvent was distilled under reduced pressure and the pure lipids obtained were stored under an atmosphere of nitrogen.

Table 1. Percentage composition and R_f values of lipids of *Basella rubra* seed oil.

Lipids	Percentage	R_f values
Hydrocarbons (HC)	2.7	0.94
Sterol esters (SE)	3.9	0.72
Triacylglycerols (TG)	59.6	0.58
Free fatty acids (FFA)	4.5	0.41
1, 3-diacylglycerols (1, 3-DG)	8.7	0.34
1, 2-diacylglycerols (1, 2-DG)	6.8	0.30
Sterols (S)	2.5	0.21
Monoacylglycerols (MG)	7.4	0.15

Separation and identification of lipid classes

The neutral and polar lipids were separated on 0.5 mm thick TLC plates using hexane:ether:acetic acid (80:20:2, v/v) and chloroform:methanol:30% ammonium hydroxide:water (60:35:5:2.5, v/v) solvent systems respectively [11]. The different components of the lipids were identified by comparing their R_f values with the standards and then verified by applying specific spray reagents [16] to the TLC plates. For quantitative determination of the lipid classes, 50 mg of the total lipids (50 µl of a 10% solution) were streaked on 20x20 cm glass plates coated with 0.5 mm silica gel G. After development, the bands were located and scraped after spraying the plates with 0.2% 2,7-dichlorofluorescein solution in methanol and viewing under UV light. The scraped bands were extracted with chloroform methanol mixture (2:1, v/v) separately. The solvent was removed under reduced pressure and the respective lipids were weighed for quantification in duplicate.

Esterification on separated lipids and purification of methyl esters

The classified lipids except hydrocarbons and sterols were esterified with born trifluoride-methanol reagent [15] for half an hour in test tubes with Teflon lined screw caps. The methyl esters so formed were extracted with hexane and purified quantitatively on TLC plates using hexane:ether (9:1, v/v) solvent system. The material (R_f 0.6) was extracted with hexane and the solvent was removed by distillation under reduced pressure to get purified methyl esters prior to the application of gas chromatography.

Table 2. Percentage composition and R_f values of polar lipids of *Basella rubra* seed oil.

Lipids	Percentage	R_f values
Phosphatidylethanolamines	1.3	0.68
Phosphatidylcholines	1.4	0.53
Lysophosphatidylethanolamines	1.2	0.46

Resolution and identification of fatty acids by gas chromatography

Methyl esters of the fatty acids were analyzed on Shimadzu GC 14A Gas Chromatograph with flame ionization detector using 1.6 x 3 mm (i.d) glass column packed with DEGS (15%) coated on Shimalite AW20 (60-80 mesh). The column temperature was programmed at 150°C for two minutes and then with a rise of 5°C/min to 200°C for 15 minutes. Injector and detector temperatures were 250°C and 300°C, respectively. Nitrogen was used as a carrier gas with a flow rate of 40 ml/min. The methyl esters were identified by comparing their retention times with those of authentic methyl esters under the same conditions. The percentages of various acids were determined by Shimadzu C-R4A Chromatopac Computing Integrator in duplicate.

Table 3. Fatty acid % composition of lipids of *Basella rubra* seed oil.

Lipids	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
SE	20.6	7.3	48.6	19.8	3.7
TG	21.2	5.9	54.1	18.5	0.3
FFA	23.4	9.6	49.4	15.6	2.0
1,3-DG	22.7	8.5	47.8	19.4	1.6
1,2-DG	24.5	7.9	52.7	14.5	0.4
MG	21.8	9.2	53.5	13.8	1.7
PE	23.9	8.3	47.6	17.3	2.9
PC	25.1	9.5	46.9	15.2	3.3
LPE	24.3	7.8	49.2	16.5	2.2

Results and Discussion

The R_f values and percentage composition of lipids of seed oil are given in Tables 1 and 2. These results show that the percentage of polar lipids is very low (3.9%) as compared to neutral lipids (96.1%) and this composition is comparable with the lipid composition of *Carum capticum* containing 4.3% polar and 95.7% neutral lipids [13]. The percentage of triacylglycerols is the highest (59.6%) among all the lipid fractions (Table 1) which is very close to the triacylglycerols of *Carum capticum* (54.1%). The other neutral components are hydrocarbons, sterol esters, free fatty acids, 1,3 and 1,2-diacylglycerols, sterols and monoacylglycerol. The polar lipids reveal the presence of phosphatidylethanolamines, phosphatidylcholines and lysophosphatidyl ehtanolamines, which are in low percentage and can be accumulated with enrichment technique for further experimental work.

The various classes of neutral as well as polar lipids except hydrocarbons and sterols were converted into their methyl esters by reacting with boron trifluoride-methanol reagent. This method is very efficient and one can work with a very small quantity of material without the loss of the material to be esterified. The methyl esters thus obtained were purified quantitatively by thin layer chromatography prior to the identification of fatty acids by GLC. The fatty acid moiety which plays a vital role in the formation of various classes of neutral as well as polar lipids was characterized by gas chromatography. The results are shown in Table 3.

The fatty acid range was C_{16} to C_{18}, containing saturated and unsaturated fatty acids in all of the lipid classes. Oleic acid ($C_{18:1}$) was predominant in neutral as well as polar lipids. *Carum capticum* fractions show a higher percentage of oleic acid [13], which is a characteristic of the Umbelliferae family. The other fatty acids were palmitic, stearic, linoleic and linolenic acids. Unsaturated fatty acids were higher as compared to saturated fatty acids in all of the lipid classes. Palmitic acids ($C_{16:0}$), which is the highest in the saturated acid profile, may be the precursor for higher fatty acids. Linoleic acid ($C_{18:2}$), which is an essential fatty acid, may be the precursor of prostaglandins (known to occur in accessory genital glands, seminal plasma and lung tissue of human body) and plays a vital role in human health [17] It may be concluded that *B. rubra* seed oil is a valuable source of both essential as well as commercially important fatty acids. This necessitates that efforts be made to process the seeds of *B. rubra* for human consumption as well as for use in soap making industry and for its medicinal value.

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SPECTROPHOTOMETRIC DETERMINATION OF METOCLOPRAM1DE HYDROCHLORIDE IN PURE AND PHARMACEUTICAL PREPARATIONS

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Abstract: Metoclopramide-HCl reacts with 2-naphthol - 3,6-disulphonic acid and sodium nitrite to give an orange color after heating for 45 s at 100°C, having maximum absorbance at 490 nm. The reaction is selective for metoclopramide-HCl with 0.01 mg/10ml as visual limit of identification and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's Law from 0.01 mg to 2.5 mg/10ml of metoclopramide-HCl and the relative standard deviation is 0.74%. The quantitative assessment of tolerable amount of other drugs is also studied.

***Keywords*:** Metoclopramide-HCl, 2-naphthol - 3,6-disulphonic acid, sodium nitrite and spectrophotometry.

Introduction

Metoclopramide-HCI is the derivative of procainamide. It is used as an antiemitic and antidopiminergic drug. It is of little benefit in the prevention of motion sickness or in the treatment of nausea, migraine and vertigo [1]. It is used for the prophylaxis of vomiting associated with cisplatin and other cancer chemotherapy. It also has cholinergic properties. The common adverse effects include bowel upset, fatigue, dizziness, restlessness, somnolence, nervousness, anxiety, dystonic reactions, parkinsonism and tardive dyskinesia, increased pituitary prolaction release, galactorrhea and menstrual disorders [2].

Many analytical techniques have been employed for the determination of metoclopramide-HCl. In the TLC [3-4] and TLC/MS [5] procedures, the stability of the color varies from five to sixty minutes [3] while hydrophilic drugs can only be detected in urine and lipophihc drugs in the liver [4]. In the HPLC [6,7,8] and capillary electrochromato-graphic [9] procedures, the mobile phase consists of methanol-water containing triethylamine adjusted to pH 4.5 with phosphoric acid [6] and a polar end capped ODS column with aqueous pH 3.0 acetonitrile for resolution prior to UV detection [7]. Also, three mobile phases for different elutions through a cis symmetry shield column are used, and the relative standard deviation is higher than 12% [8]. The analysis of basic compounds by capillary electrochromatography on silica-based materials using conventional HPLC stationary phases has failed to address the problem of severe peak tailing and non-reproducible chromatograms [9]. In the spectrophotometric procedures [10-12] and UV spectrophotometry [13], extraction in chloroform is carried out prior to the spectro-photometric determination [10], whereas the complex is stable only for two hours and the reaction is pH sensitive [12]. Furthermore, more robust stability indicating methods are required to confirm the results [13]. Long and tedious procedures are followed in x-ray diffraction [14] and proton NMR spectroscopy [15]. During a systematic study of drugs of abuse [16-

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19], it was found that metoclopramide-HCl reacts with 2-naphthol-3,6-disulphonic acid and sodium nitrite to give an orange complex having maximum absorbance at 490 nm. The reaction obeys Beer's Law and has 0.01 mg /10ml as visual limit of identification. The color reaction has not been reported in the literature. The present method is simple, accurate, precise and sensitive. Percentage of tolerable limits of other drugs not interfering is also studied.

Materials and Methods

Apparatus and reagents

Hitachi U-1100 spectrophotometer with 1.0 cm silica cells was used to measure the absorbance and graduated pipettes were employed. Analytical grade chemicals and doubly distilled water were used. Metoclopramide-HCl (Pacific Pharma) standard solution (w/v, 1.0 mg/ml) was prepared in distilled water to get a stock solution, which was diluted further as required, while 0.1% (w/v) 2 naphthol-3,6-disulphonic acid and 0.1% (w/v) sodium nitrite were prepared in distilled water.

General procedures

To an aliquot of metoclopramide-HCl containing 0.01mg to 2.5 mg/10ml was added 3 ml of 0.1% 2-naphthol- 3,6-disulphonic acid, 0.07 ml of 0.1% of sodium nitrite and the contents were heated for 45 s in a water bath at 100°C, and the volume was made up to 10 ml with distilled water. The resulting absorbance of the orange color was measured at 490 nm employing all reagents except metoclopramide-HCl as a blank. The experiment was repeated with different volumes of standard metoclopramide-HCl solution and a calibration curve was prepared (Fig.1). The color reaction obeys Beer's Law from 0.01 to 2.5 mg/10ml of metoclopramide-HCl.

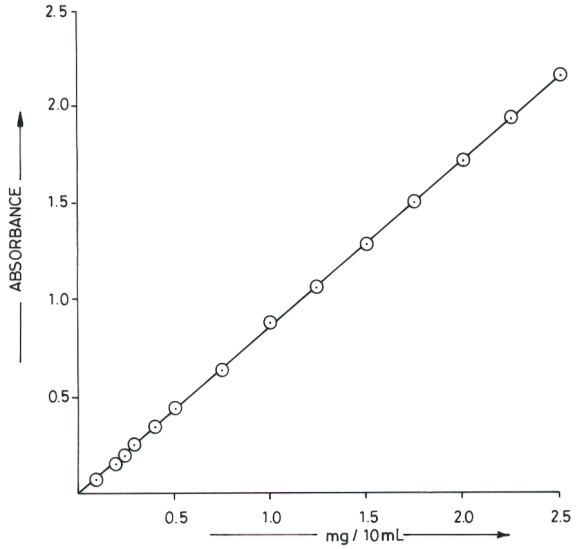

Figure 1. Calibration curve of metoclopramide with 2-naphthol, 3, 6-disulphonic acid.

Procedure for studying the interfering compounds

To an aliquot containing 1mg/ml of metoclopramide-HCl, different amounts of various compounds (1mg/ml) (w/v) were added individually until the solution showed the same (±0.01) absorbance as that of pure metoclopramide-HCl solution without the addition of the organic compound, under experimental conditions, as described in the general procedure, The value was calculated as the percentage of organic compound with respect to the amount of metoclopramide-HCl.

Procedure for the determination of metoclopramide-HCl in pharmaceautical preparations

Tablets containing 10 mg of metoclopramide-HCl were powdered, weighed, dissolved in distilled water and filtered. The filtrate was diluted to get a 1 mg/ml (nominal) solution of metoclopramide-HCl. An aliquot containing 0.01 mg to 2.5 mg/10ml was taken and the procedure as described above was followed. The absorbance was measured at 490 nm. The quantity per tablet was calculated

from the standard calibration curve.

Syrup containing 5mg/5ml of metoctopramide-HCl was weighed, dissolved in distilled water and filtered. If turbidity persisted, the contents were centrifuged until a clear supernatant was obtained. After filtration, a 1.0 mg/ml (nominal) solution of metoclopramide-HCl was prepared. An aliquot containing 0.01 mg to 2.5 mg/10 ml was taken, the above procedure was followed and the absorbance was measured at 490 nm. The quantity of metoclopramide-HCl per 5 ml of syrup was calculated from the calibration curve.

The same procedure as for tablets, was adopted if the contents were in powder form but if the contents were in a liquid form, then a 1 mg/ml solution with respect metoclopramide-HCl was prepared directly in distilled water. The above procedure was followed using an aliquot containing 0.01 to 2.5mg/10ml of metoclopramide-HCl and the absorbance measured at 490 nm. The quantity of metoclopramide-HCl per injection was calculated from the standard calibration curve.

Results and Discussion

Absorption spectrum of the colored complex

Metoclopramide-HCl reacts with 2-naphthol - 3,6-disulphonic acid and sodium nitrite when heated for 45 s at 100°C to give an orange colored complex, the absorption spectra of which under optimum condition lies at 490 nm (Fig. 2).

Effect of color producing reagent

There are two color-producing reagents: 2-naphthal-3, 6-disuiphonic acid and sodium nitrite. It was found that 0.003 mg/10 ml of 0.1% 2-naphtnof 3,6-disulphonic acid (Fig. 3) and 0.00007 mg/10 ml of sodium nitrite gave maximum color (Fig. 4). If the concentration of these reagents was changed, the color intensity decreased and the color became unstable and if either one of the reagents was absent, then too, the color did not develop. The probable mechanism of the color reaction may be diazotization. A variety of organic compounds form diazonium ions [20] when they interact with sodium nitrite, which are water-soluble due to SN-1 mechanism, 2-naphthol-3, 6-disulphonic acid being one of them. Generally these complexes cannot be isolated in pure state at ordinary temperatures as they exist only in solution in equilibrium with the other components. They can however be detected readily because of their absorption spectra, as the rate of formation of complexes in solutions is generally rapid (45 s in the present color reaction). Changes in spectrum or color are associated with a transfer/interchange of electrons when such molecules undergo properly oriented collisions. Thus metoclopramide-HCl acts as an efficient n-electron donor, not only due to its aromatic ring but also being a nitrogen base. It has a non-bonded electron pair available for co-ordination with 2-naphthol-3, 6-disulphonic acid after its oxidation by sodium nitrite forming a stable orange complex having maximum absorbance at 490nm.

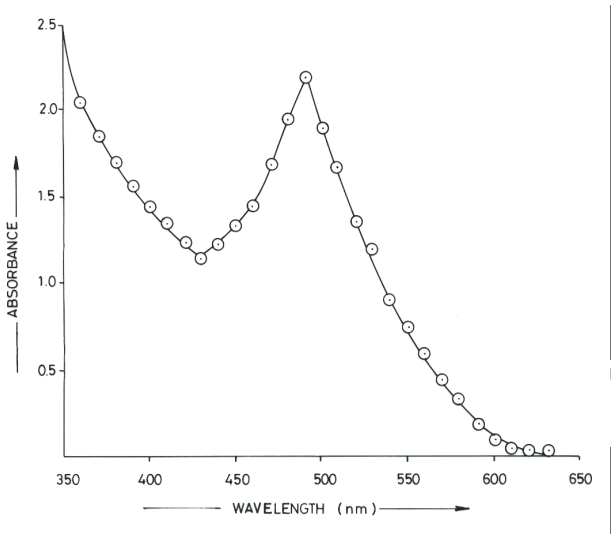

Figure 2. Absorption of spectrum of metoclopramide hydrochloride with 2-naphthol 3, 6-disulphonic acid.

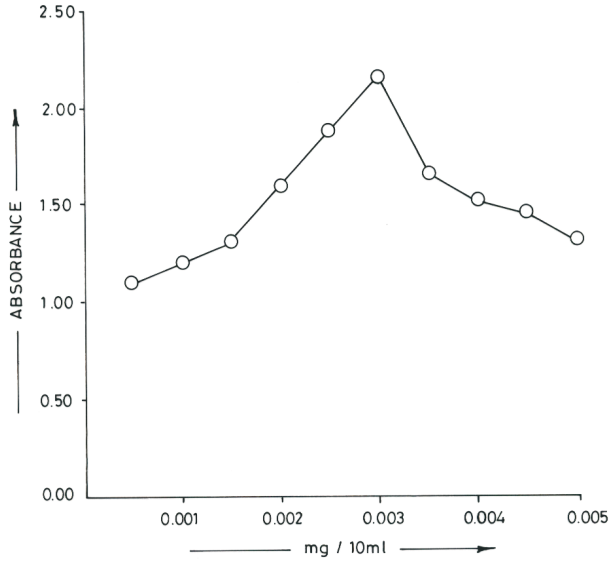

Figure 3. Effect of 2-naphthol 3, 6-disulphonic acid.

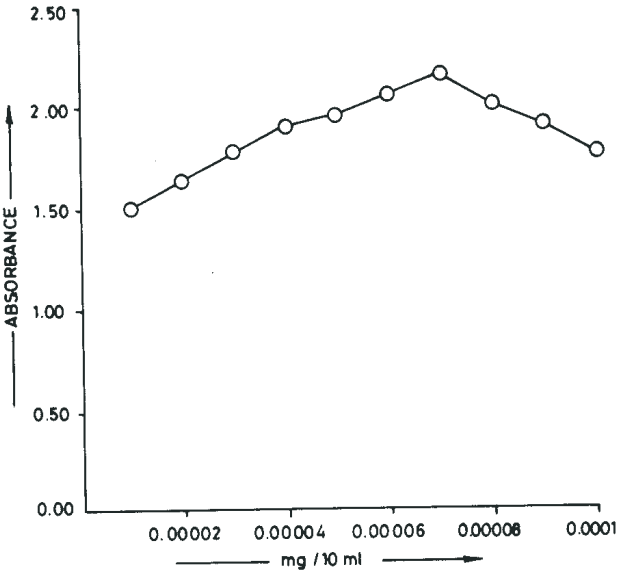

Figure 4. Effect of sodium nitrite.

Effect of temperature and heating time

The effect of temperature is shown in Fig. 5. With the rise of temperature, the color intensity increased and was stable at 100°C. The color developed at room temperature but was unstable and of less intensity. The absorbance of the developed color was stable for more than 24 h. A waterbath was used to carry out the temperature studies. The effect of heating time on color intensity is shown in Fig. 6. It was found that heating for 45 s at 100°C gave maximum color; above

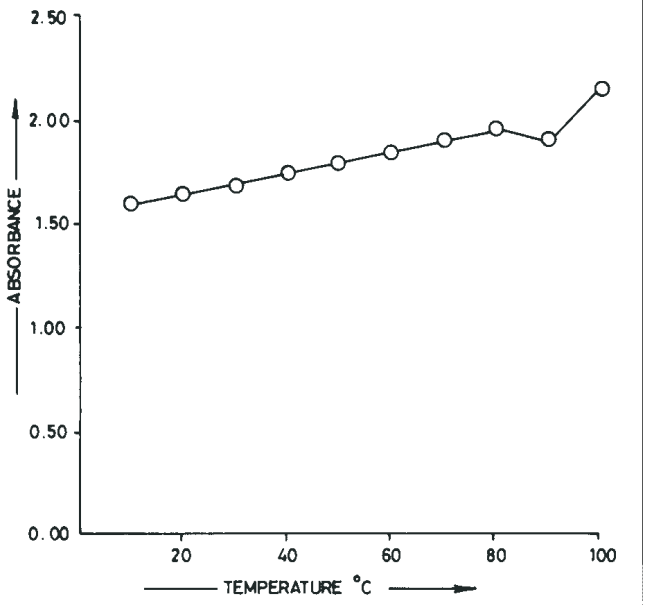

Figure 5. Effect of temperature.

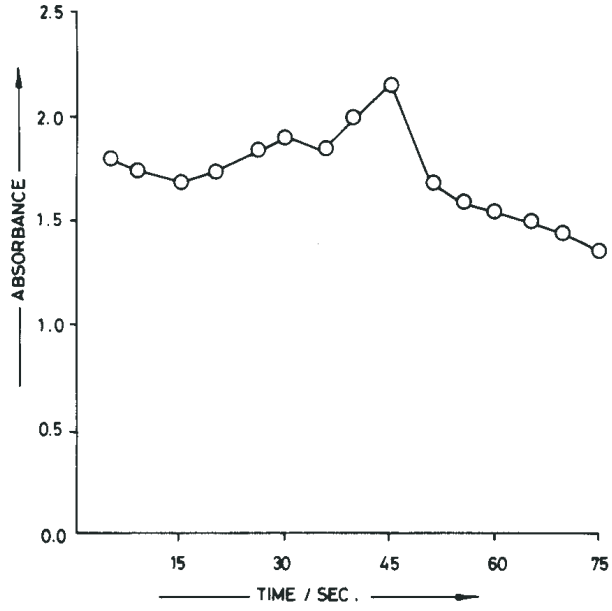

Figure 6. Effect of heating time.

and below this time the color intensity decreased and was unstable.

Effect of organic solvents

Different organic solvents such as choloroform, n-hexane, xylene, acetone, benzene, dichloromethane, dioxane, formaldehyde and tetra hydrofurane, were tested for color extraction and for stability but none was effective and therefore no organic solvent was employed.

Sensitivity

The results for the determination of metoclopramide-HCl are shown in Tables 1 and 2, which reveal the sensitivity, validity and repeatability of the method. The method is also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found by the above procedure does not exceed the relative standard deviation of 0.74%, which is the replicate of five determinations (Table 1). The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 0.01mg to 2.5mg/10ml. The apparent molar absorptivity calculated was 0.3714 x 10 ^{4} and the regression equation [21] was calculated by the method of least squares from nine points, each of which was the average of five determinations. The correlation between absorbance and concentration is 0.999 in terms of correlation coefficient (r).

Table 1. Determination of metoclopramide – HCl from pure solution.

Metoclopramide-HCl taken (mg/10ml)	Metoclopramide-HCl *found (mg/10ml)	Relative Standard Deviation %
0.10	0.134	0.74
0.20	0.190	0.52
0.30	0.220	0.45
0.40	0.395	0.25
0.50	0.410	0.25
1.00	1.134	0.74
1.50	1.493	0.07
2.00	2.010	0.05
2.50	2.5	0.04

*Every reading is an average of five readings.

Table 2. Optical characteristics, precision and accuracy of the proposed method.

Parameters	Values
λ_{max} (nm)	490
Molar Absorptivity ($mol^{-1}cm^{-1}$)	0.3714 x 10^4
Regression Equation (Y)*	
Slope (b)	0.8630
Intercept (a)	0.0025
Correlation Coefficient (r)	0.999
Relative Standard Deviation (RSD%)**	0.74%
% Range of Error (Confidence Limit) at 95% Confidence Level.	10±0.025

* Y=a+bC where C is the concentration of analyte (mg/l0ml) and Y is the absorbance unit.

** Calculated from five determinations.

Interference

The quantitative assessment of tolerable amount of different organic compounds under the experimental conditions is given in Table. 3. Various amounts of diverse interfering compounds were added to a fixed amount of metoclopramide-HCl(1mg/ml) and the recom-mended procedure for the spectrophotometric determination was followed. Other common interferences, like buscopan, zantac, septran, cimet, semidine and glucophage did not interfere.

Table 3. Quantitative assessment of tolerable amount of other drugs.

Drugs	Maximum Amount Not Interfering* (%)
Buscopan	100
Zantac	50
Septran	100
Cimet	100
Barbituric Acid	50
Semidine	20
Marzine	200
Glucophage	40
Dextropropoxyphene –HCl	100
Dextromethorphane – HBr	50
Sulpiride	50
Chlorpheneramine Maleate	150
Morphine	100
Paracetamol	150
Fluoxetine	200
Aspirin	100
Napro xen	250
Nicotinic acid	150
Aldomet	200
Atenolol	100

*The value is the percentage of the drug with respect to 1 mg/10ml of metoclopramide – HCl that causes + 0.01 change in absorbance.

Application

The proposed method is successfully applied for the quality control of pure metoclopramide-HCl and in the pharmaceutical dosage form, as shown in Table 4.

Conclusion

The spectrophotometric method for the determination of metoclopramide-HCl is simple, reliable, sensitive and less time consuming. The statistical analysis is in good agreement with those of the official British Pharmacopeia 1988. The color reaction is selective for metoctopramide-HCI. The method can be successfully applied to the micro determination of metoclopramide-HCl either in pure, or in pharmaceutical preparations. The color reaction has 0.01mg/10ml as the visual limit of identification. The advantage of the present procedure is that it does not require many solvents, whereas the HPLC procedures [6-8], are long, tedious and expensive, involving many reagents and solvents showing high RSD value i.e. 12% [8], and the color stability varies from five to sixty minutes in the TLC procedure [3]. The literature indicates that this color reaction has not been reported previously. The present method is precise, accurate and other compounds like buscopan, zantac, septran, cimet and semidine do not interfere. A significant advantage of a spectrophotometric determination is its application to the determination of individual compounds. This aspect of spectrophotometric analysis is of

Table 4. Determination of Metoclopramide-HCl from pharmaceuticals preparations.

Drug	Trade Name	Pharmaceutical preparations	Amount Present (Manufacturer's Specifications) (mg)	Amount found*(mg)	Percentage recovery (%)
Metoclopramide-HC1	Metoclon (Sami Labs, Pakistan)	Tablet	10	9.96	99.6
Metoclopramide-HC1	Maxolon (Smith Klin Beecham, Pakistan)	Tablet	10	10.01	100.01
Metoclopramide-HC1	Maxolon (Smith Klin Beecham, Pakistan)	Syrup	5 mg /5ml	4.95mg/5ml	99.0
Metoclopramide-HC1	Elpomide (Elite Pharama, Pakistan)	Injection	10mg/2ml	10mg/2ml	100

* Every reading is an average of five determinations

major interest in analytical pharmacy, since it offers a distinct possibility of quality control in the assay of pharmaceutical dosage formulations.

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CONTROLLING INCLUSIONS THROUGH FILTRATION IN INVESTMENT CASTING PROCESS

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Abstract: A technique for the placement of a ceramic foam filter in the feeding up of investment mould was developed which proved quite efficient in removing smaller and major inclusions through various filtration modes. Contaminated old aluminum scrap was used to prepare the melt without the addition of any cleansing and covering fluxes and the main reason was to produce more and more inclusions. Vigorous stirring was also intentionally carried out to form as much oxides as possible. During present research work effective filtration was observed. No leakage through sides of the filter occurred and similarly no choking was seen during feeding of molten metal. Microstructural studies showed the maximum retention of inclusions not only on the surface of filters but also within the various channels of the main body of the filter. The microstructures taken from the filtered test pieces were free from inclusions, which showed the effectiveness and proper placement of the filter.

Keywords: Aluminum scrap, ceramic foam filter, inclusions, microstructural study

Introduction

Inclusion removal processes play a key role in casting quality control and the use of ceramic foam filters has grown substantially over the past few years [1,2,3]. The occurrence of non-metallic inclusions in metal castings is a serious cause of scrap. When melting aluminum and its alloys, an aluminum oxide film is formed on the surface of the metal. This skin is effective in preventing further oxide formation. However, oxide is formed every time fresh metal is exposed to the surrounding air. In addition every piece of aluminum charged into the furnace has a thin surface film of oxide which adds to the oxides already present. The aluminum oxide introduced into the metal has a similar density to the parent metal and does not separate out on the top of the melt. These oxide particles can therefore be carried over into the actual casting, giving rise to oxide or dross inclusions, which is a frequent cause of casting scrap. The oxides present in aluminum are porous and have a tendency to harbor entrapped gas. They also have a tendency to act as nuclei for hydrogen bubble formation [4]. The elimination of oxides in aluminum has become more important as the demand for high quality castings increases. The results of inclusion-contaminated melt are many: poor machinability, increased porosity, reduced corrosion resistance, loss of mechanical properties, lack of pressure tightness, surface finish (unsuitable for anodizing and perhaps even for painting) and reduced fluidity. In other words, inclusions in aluminum can have a profound impact on casting scrap.

Melt inclusions can be formed by a variety of factors, including salts, fluxes, grain refiners, eroded moulding materials and refractories [5,6]. There are, however, methods to clean out inclusions prior to casting. Therefore, filtering of the liquid metal as it

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enters the mould is extremely important [7,8,9]. Although the various filter media have been successfully used particularly in sand casting productions, it is difficult to place the filter in investment moulds. Useful guidelines have not yet been set on the application of filters in investment moulds, for example effective setting of the filter, filter pore size and effective filter area, selection of the correct filter for the proper flow rate and also for a particular type of inclusions. On the basis of this, further research work is required to produce quality castings.

Investment casting process produces complex, high value added components for a variety of specialized industries including aerospace, power generation, nuclear, medical and automotive [10]. Due to better improvements, the investment industry is becoming increasingly more aware of the potential for research and the development of new processes in order for the industry to remain competitive in the new Millennium.

The objective of present work was to prepare investment moulds incorporating ceramic foam filters and to clean various heats of aluminum alloys using such moulds. The objective also included metallography of filtered metal as well as the microscopy of the used filter section for better understanding and the examination of inclusions retained on the filter surfaces.

Materials and Methods

Production of wax patterns for the preparation of investment moulds

Wax patterns were produced using a split die made of pure aluminum. The die was lubricated with silicone spray and was bolted. Liquid wax was then introduced under pressure into the die from the injection nozzle of the machine. The wax was injected into the die at a temperature of 60°C. After about 10 seconds, the die was opened and the wax pattern was removed.

Wax runners were prepared in a similar manner. However a steel rod with threads at one end was placed into the die before injecting the wax. The threaded end was kept outside the die and was used for bolting the hanger. Two halves of the metallic die of the runner are shown in Fig. 1.

Figure 1. Two halves of the metallic aluminum die of runner.

Pattern assembly

The wax patterns were assembled using heated wax as an adhesive and the resultant assembly of wax patterns is known as “tree”. Completed wax pattern tree assemblies are shown in Fig. 2.

Figure 2. Photograph showing the wax patterns assembly incorporating the patterns, the runner and pouring cup.

Washing

The trees were washed by dipping in liquid xylene, which removed the residual lubricant and dust particles. Xylene also slightly etched the wax surface of the investment, which causes better adherence of the refractory mould coating on the wax. The washed trees were subsequently dried in air.

Ceramic shell investment

Coating the wax pattern trees with a ceramic refractory shell involved the following sequence of operations:

1. Application of slurry
2. Application of refractory materials
3. Drying

The finished mould shell was obtained by repeating the above sequence several times to achieve a refractory coating of sufficient thickness (6-12 mm) around the wax pattern.

Application of slurry

The pattern assembly, incorporating the pattern, the running and feeding system and the pouring cup were dipped in the slurry 'A' as a single unit, while the container was revolving at 24 rpm. Table 1 shows the composition of various components used in the slurry.

Table 1. Composition of slurry 'A'.

Component	Quantity
Colloidal silica solution	10 L
Water	4 L
Zircon flour, 325 mesh	45 kg

The whole tree was dipped 2-3 times in slurry 'A' except the cup of the running system.

Application of refractory materials

After draining off the excess slurry from the tree, the wet layer was immediately stuccoed with refractory aggregates. The primary stucco consisting of zircon flour (325 mesh) was applied by a raining technique. In this technique the assembly was placed in a stream of refractory particles. In this way the refractory particles were stuck to the wet slurry. After that the coating was dried as mentioned below.

Drying

The coating was allowed to dry in air for 6 hours. After drying, the first coat was completed. The same procedure was repeated for the 2nd coat and the same slurry and stucco material were applied. The tree was then allowed to dry for 6 hours in air.

For the third coat, the tree was dipped into the same slurry but stucco material was different. This time it was stuccoed through fluidized bed of mullite (47-50 mesh). For the fourth coat the tree was immersed in slurry B, the composition of which is given in Table 2. After draining off the excess slurry from the tree, the stucco material, mullite (47-50 mesh), was applied through fluidized bed. The tree was then dried for 6 hours at room temperature. The 5th coat was carried out by immersing the tree into the slurry 'B' followed by the application of the same stucco material with 22-47 mesh in fluidized bed and allowed to dry for 6 hours at room temperature. The conditions for 6th coat were the same as for the 5th coat. Finally, the trees were dried for 36 hours at room temperature. The wax assembly after final coating is shown in Fig. 3.

Table 2. Composition of slurry 'B'.

Component	Quantity
Colloidal silica solution	10 L
Water	4 L
Mullite (60-210 mesh)	45 kg

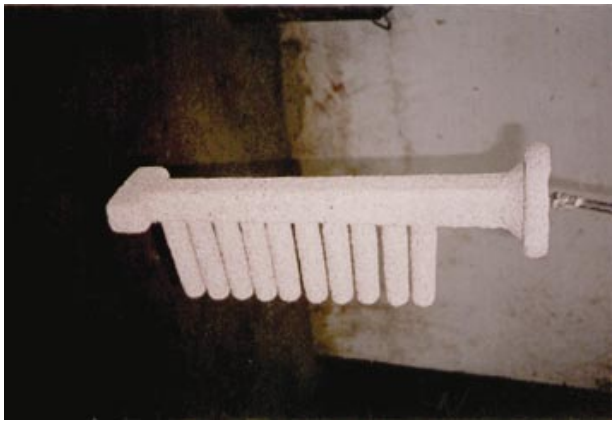

Figure 3. Wax assembly after coating.

Dewaxing

Dewaxing was carried out in a gas-fired furnace. The furnace was constructed of mild steel case lined with firebricks. Air-dried trees were hung upside down on wheeled trolleys, running on rails, inside the furnace.

A flash dewaxing method was used to remove the wax from the ceramic shells. This method involved heating the inverted shell rapidly by means of gas burners. The dewaxing furnace operated at a temperature of 300°C and the dewaxing cycle lasted 60 to 100 minutes. The expelled molten wax was collected in water trays situated under the bottom doors of the furnace. After melting as much wax as possible out of the refractory mould, the shells were fired at about 1000°C to remove any final traces of wax. The trees usually catch fire at this temperature. The trees along with the trolley were then cooled to room temperature. The fired moulds were then removed from the trolley and inspected. The moulds were then given a final dip coat in slurry 'B' to bind any loose sand and to seal any invisible cracks. The moulds were then dried for 24 hours at room temperature before use.

Preheating and Firing

Before pouring, the moulds were preheated and fired to 1000-1050°C for half an hour to

- remove any final traces of wax
- remove moisture
- help maintain the fluidity of the metal and assist with the running of thin section
- minimize the size of risers

Placement of ceramic filter in investment mould

The filter was seated and fastened into the pouring cup of the running and feeding system of the mould by using slurry consisting of colloidal silica solution, water and zircon flour in semi solid form. The mould, incorporating the filter was then dried and fired at 1000-1050°C again for half an hour (Fig. 4).

Figure 4. A feeding cup separated from the gating system of 'tree' showing the top view of the filter placement.

Melting Practice

An aluminum silicon alloy close to composition of standard BS 1490 LM-13 alloy from the light metal series was prepared. The various heats of this alloy were prepared using dirty and rusty recirculated scrap. The scrap consisted of old defective castings of pistons, runners, risers and pouring basins. However the various additions in the form of master alloys were made to adjust the composition of the present alloy.

A gas fired crucible furnace was used for the preparation of various heats. No fluxing

treatment was given to the molten metal. The molten metal was stirred vigorously to form maximum inclusions within the melt. At about 730°C, furnace heating was stopped and the crucible was taken out from the furnace. At this stage degassing was carried out by plunging the degassing tablet into the molten metal by a bell type plunger and the slag formed on the surface was dissolved into the molten metal by continuous stirring action using a rotary impeller unit. The molten metal was then poured into investment moulds in red-hot state to avoid choking of the filter and to ensure complete filling of the mould.

Selection of sample for microscopy

The samples for microscopy were prepared in three ways namely:

1. From the surface of used filter to examine the cake type filtration
2. From the main body of the used filter to observe deep bed filtration
3. From the filtered casting

In addition to the above, microscopy of as received (unused) filter was also carried out.

Optical and scanning electron microscopy

All samples were machined and mounted in the required size using mounting resin. The specimens were hand ground and polished using conventional metallographic techniques.

Results and Discussion

During the present work, a technique for the placement of a 20-pore per linear inch (20 ppi) ceramic foam filter in the feeding cup was developed. The conventional gating system incorporating ceramic foam filter is difficult to adopt in investment casting technique due to the complex nature of the tree. However, the same may be successfully adopted in other casting methods such as sand casting and die casting techniques [1,2].

During the present research work, the technique adopted was quite efficient in removing inclusions from the melt. The castings, free from inclusions, were successfully prepared. The filter worked efficiently and no choking was observed.

Various heats of the alloy were prepared. However, the chemical composition of only a representative heat is shown in Table 3.

Table 3. Chemical analysis of investment cast sample.

Description	Element, Wt %							
	Si	Mn	Fe	Ni	Cu	Zn	Mg	Al
Alloy prepared	11.6	0.2	1.1	1.0	1.2	0.1	1.0	Balance

SEM microstructure of as-received (unused) filter is shown in Fig. 5. Fig. 6 shows the surface of a used filter. The micrograph shows many inclusions, which are captured by the filter. A similar microstructure at high magnification is shown in Fig. 7. Actually the surface of unused filter not only contains the major cavities but also the micro porosity which is quite helpful in removing smaller inclusions because these are captured along with bulky inclusions on the surface. This point may be clearly understood if we look at the micrograph shown in Fig. 8. The microporosity of the unused filter surface is clearly visible in this microstructure. However the bulky inclusions larger than the opening of the filter cavities are dispersed on the whole surface of the filter as shown in Fig. 9. This phenomenon is categorized as cake mode filtration and is explained elsewhere [11]. Deep bed filtration mechanism was also observed during the present work as shown in Fig. 10. Similarly, Fig. 11 shows a pin-type oxide inclusion along with other smaller inclusions. According to this mechanism, smaller inclusions go inside the filter surfaces through the major cavities of the filter.

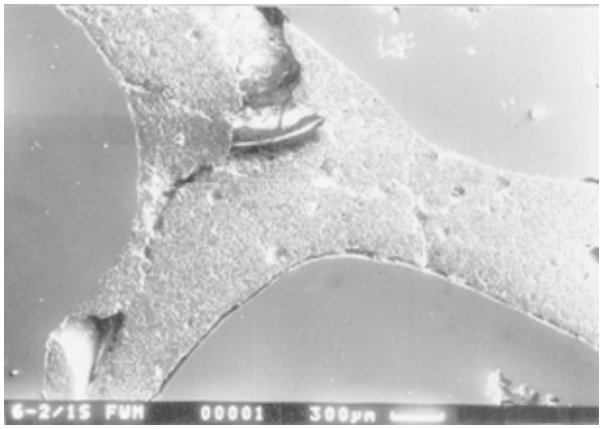

Figure 5. Microstructure of as-received 20 ppi ceramic foam filter.

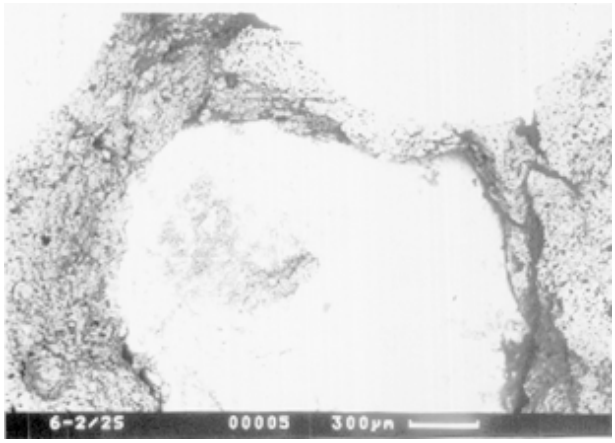

Figure 6. Microstructure of used filter showing many inclusions.

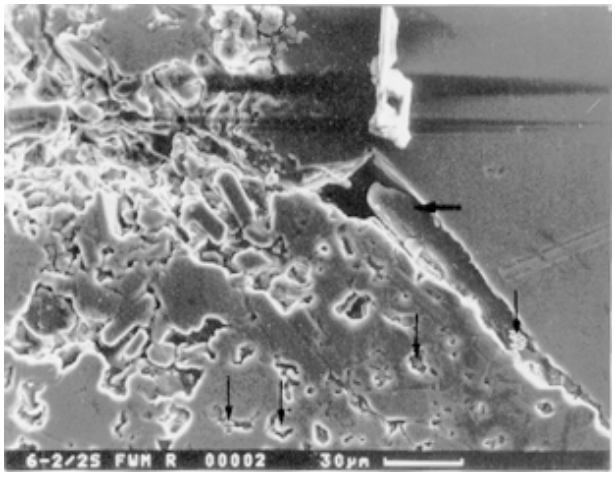

Figure 7. Microstructure showing entrapped particles indicated by arrows on the surface of a used filter.

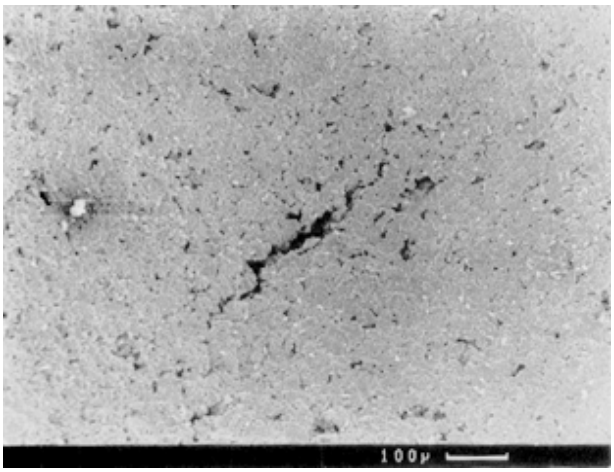

Figure 8. Microstructure showing the surface in between the main pores of as- received filter. Porosity is clearly visible at many places

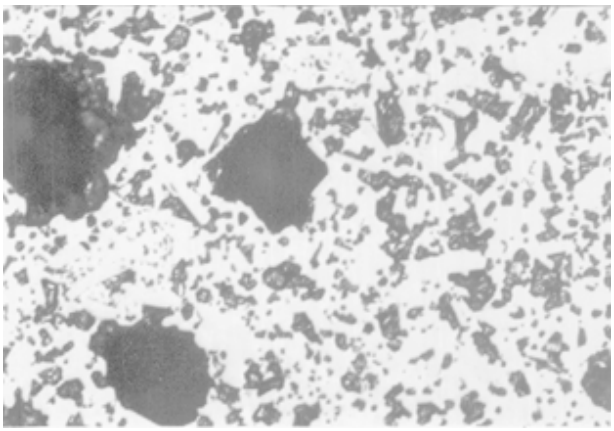

Figure 9. Microstructure showing a lot of inclusions on the entire surface of used filter.

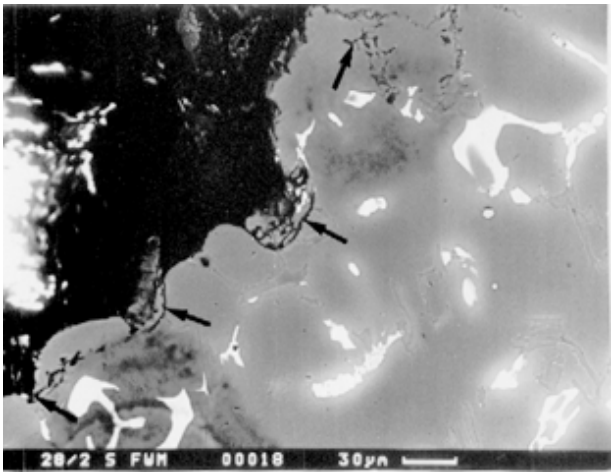

Figure 10. Microstructure showing oxide particles indicated by arrows along the filter side.

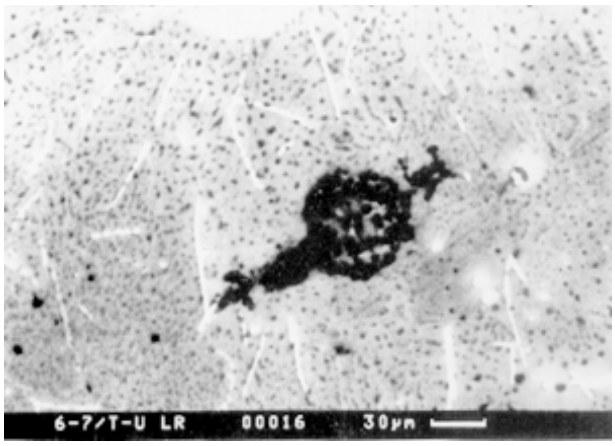

Figure 11. Microstructure showing a pin type oxide inclusion alongwith other smaller inclusions.

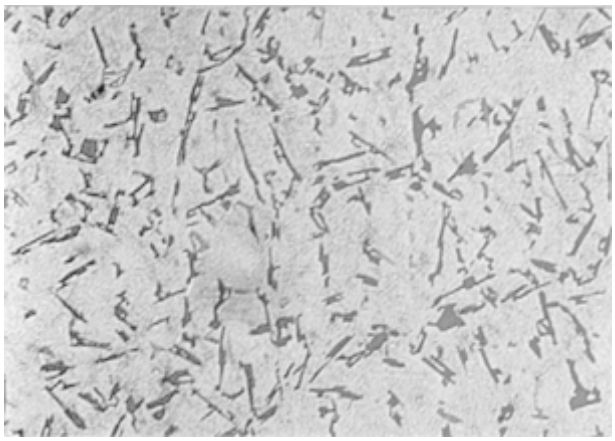

Figure 12. Microstructure of a filtered test piece showing silicon needles and particles in a matrix of aluminum. No inclusions are visible (at X-100).

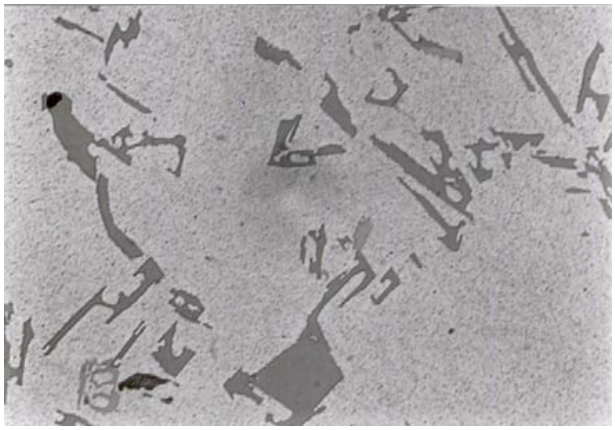

Figure 13 . Similar microstructure as shown in Fig. 12 at high magnification (X-200). Again no inclusions are seen.

During the present study, a 20 ppi depth filter was used which in fact is a ceramic form filter where the inclusions are dispersed through part or all of its volume (depth). It thus has the advantage of having a larger surface area for entrapment and can trap particles much smaller than the pores present in the filter bed. At high temperature, these inclusions sinter to the filter surface. The inclusions are transported to the filter surface by diffusion, sedimentation, impingement and other hydrodynamic effects and their capture occurs due to surface forces developed through pressure or chemical effect [11]. Apelian and Shivkumar [9] have reported similar observations.

The microstructure of a representative sample taken from a filtered test piece is shown in Fig. 12. The structure is free from inclusions. This micrograph shows silicon needless and particles in a matrix of aluminum at X-100. Another microstructure of a representative sample after filtration at X-200 is shown in Fig. 13. Again no inclusions were observed and the structure contains regular features of silicon plates in aluminum matrix.

By careful examination of these two microstructures, it was observed that the molten metal after passing through the filter contained no foreign particles/inclusions and the particles/ inclusions were held either on the filter surface or entrapped within the main body of the filter.

Conclusion

The presently developed technique was quite efficient in removing foreign particles/ inclusions. The technique is useful for economical production of premium quality clean castings as very fine inclusions alongwith larger particles can be successfully removed using ceramic foam filter.

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ALGEBRAIC APPROXIMANTS TO *EXP(Z)* AND OTHER FUNCTIONS

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Abstract: We show that the algebraic approximants for the function e^z can be easily obtained by using Pade′ approximants to the function $ln(1 + x)$: If $f(0) = 0$ then an algebraic approximant can be found with the help of a Pade′ approximant for $f^{-1}(x)$.

Keywords: Algebraic approximant, Pade′ approximant

Introduction

Recently Gao and Wang [1] have discussed an approximation scheme called approximation by algebraic functions. Let $F(z,w)$ be a polynomial of degree n in z and degree m in w,

$$F(z,w) = \sum_{i=0}^{n} \sum_{j=0}^{m} a_{ij} z^i w^j . \quad (1)$$

A function $w = \phi(z)$ is defined to be an *algebraic function* of order [n,m] if it satisfies

$$F(z, \phi(z)) \equiv 0. \quad (2)$$

Further an algebraic function is called an *algebraic approximant* of order [n,m] to a function $f(z)$ if

$$F(z, f(z)) = O(z^{m+n+1}). \quad (3)$$

Gao and Wang [1] have shown that the algebraic approximants to *exp(z)* of order [1,m], for various values of m, can be used to develop difference schemes for the numerical solution of an ordinary differential equation of order one. Their method essentially consists of replacing *exp(z)* by its Maclaurin series in Eq.(2) and matching coefficients of powers of z upto the desired order. As an example of their results, we quote the following algebraic approximant to *exp(z)* of order [1,3].

$$(11+3z) + (27+27z)e^z + (-27+27z)e^{2z} + (-11+3z)e^{3z} \equiv 0 + O(z^7). \quad (4)$$

In this paper we will show that their results can be obtained in a systematic manner by employing the Pade′ approximants for the function $ln(1+ z)$. For the theory of Pade′ approximation, see Baker [2]. Pade' approximants for the functions *exp(z)* and *tan(z)* were found by Ahmad [3] where the coefficients were shown to depend on the values of the Legendre polynomial, $P_n(x)$, and its derivatives at $x = 1$.

Algebraic Approximants to *exp*(*z*)

The function $ln(1+x)$ has the well-known expansion for $-1 < x \leq 1$,

$$ln(1 + x) = \sum_{n=0}^{\infty} = \frac{(-1)^n x^n}{n}$$

It is easily established that

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$$\ln(1+x) = \frac{2x}{2+x} + O(x^3), \quad (5)$$

$$ln(1+x) = \frac{6x+3x}{6+6x+x^2} + O(x^5), \quad (6)$$

and

$$ln(1+x) = \frac{60x+60x^2+11x^3}{60+90x+36x^2+3x^3} + O(x^7). \quad (7)$$

The rational functions in Eqs. (5)-(7) are respectively the [1,1], [2,2] and [3,3] Pade′ approximants for $ln(1+x)$. The [n,n] Pade′ approximant is defined by the relation

$$ln(1+x) = \frac{a_0 + a_1x + \ldots + a_mx^n}{b_0 + b_1x + ::: + n_nx^n} + O(x^{2n+1}) \quad (8)$$

Let $1+x = e^z$, then $ln(1+x) = z$ and (8) becomes

$$z = \frac{a_0 + a_1(e^z-1) + \ldots + a_n(e^z-1)^n}{b_0 + b_1(e^z-1) + \ldots + b_n(e^z-1)^n} + O(e^z-1)^{2n+1}). \quad (9)$$

Writing the numerator as well as the denominator of the above result as a polynomial in e^z, the above result is seen to be equivalent to

$$z = \frac{c_o + c_1e^z + \ldots + c_ne^{nz}}{d_0 + d_1e^z + \ldots + d_ne^{zn}} + O(z^{2n+1}), \quad (10)$$

where c_i, d_i i = 1,…, n are easily calculated in terms of a_i and b_i, if we rewrite (10) in the form

$$(-c_0+d_0z)+(-c_1+d_1z)e^z+\ldots +(-c_n+d_nz)e^{nz} = O(z^{2n+1}), \quad (11)$$

we get the left hand side of (11) as the algebraic approximant to *exp(z)* of order [1,n].

Let $e^z = 1+x$ successively in Eqs. (5)-(7). We obtain

$$z = \frac{-2+2e^z}{1+e^z} + O(z^3), \quad (12)$$

$$z = \frac{-3+3e^{2z}}{1+4e^z+e^{2z}} + O(z^5), \quad (13)$$

$$z = \frac{-11-27e^z+27e^{2z}+11e^{3z}}{3+27e^z+27e^{2z}+3e^{3z}} + O(z^7). \quad (14)$$

Eq. (14) reproduces the above quoted result (4) of Gao and Wang [1] and Eqs. (12) and (13) are respectively equivalent to the algebraic approximants to *exp(z)* of order [1,1] and [1,2] as reported in [1].

Algebraic Approximants to Other Functions

It has been noted by Gao and Wang [1] that the algebraic approximant for a function $f(x)$ of order [n,1] is simply the [n,n] Pade' approximant for that function. We wish to point out the fact that the algebraic approximant of order [1,n] has a similar relationship with the function $f^{-1}(z)$ provided (i) $f(0) = 0$, (ii) the inverse function $f^{-1}(x)$ exists, and (iii) $f^{-1}(x)$ possesses a Pade′ approximant.

Let

$$f^{-1}(x) = \frac{a_0 + a_1x + \ldots + a_nx^n}{b_0 + b_1x + \ldots + b_nx^n} + O(x^{2n+1}) \quad (15)$$

Let $f^{-1}(x) = z$. Eq. (15) becomes

$$z = \frac{a_0 + a_1w + \ldots + a_nw^n}{b_0 + b_1w + \ldots + b_nw^n} + O(w^{2n+1}) \quad (16)$$

where, for simplicity, we have used the symbol w for $f(z)$. Since $f(0) = 0$, $O(w^{2n+1})$ may be replaced by $O(z^{2n+1})$ in (16) and the resulting expression is equivalent to the algebraic approximant of order [1,n] for $f(z)$. We were not able to use this method in Section 2 because the function $ln(x)$ which is inverse to e^x fails to satisfy conditions (i) and (iii). As an example, consider the [4,4] Pade′ approximant for the function $\tan^{-1}(x)$

$$\tan^{-1}(x) = \frac{x(35 + \frac{35}{3}x^2)}{35 + 30x^2 + 3x^4} + O(x^9). \qquad (17)$$

We remark that the denominator in (17) can be expressed as $x^4P_n(1/ix)$ or, equivalently, changing all negative signs in $P_n(x)$ to positive and re-writing the coefficients in the reverse order [3]. Now defining $z = \tan^{-1}(x)$, Eq. (17) transforms to

$$z = \frac{\tan z(35 + \frac{55}{3}\tan^2 z)}{35 + 30\tan^2 z + 3\tan^4 z} + O(z^9). \qquad (18)$$

Letting $w = \tan z$, we obtain from (18)

$$35z - 35w + 30zw^2 - \frac{55}{3}w^3 + 3zw^4 = O(z^9),$$

as the [1,4] algebraic approximant to the function $\tan z$.

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EFFICIENCY AND BIAS REDUCTION IN RATIO ESTIMATION UNDER A MODEL

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Abstract: An almost unbiased ratio-type estimator based on mean and Sahoo's [8] estimator is suggested. Its comparison is made with mean estimator, usual ratio estimator and two Chakrabarty's [2] estimators in respect of bias and mean square error (*MSE*) under a model. The modified estimator is found to be more attractive because of it being more efficient and almost unbiased. To observe the validity of estimator, we obtained the results numerically in Tables 1 through 4. By observing these tables, one can find that the suggested estimator is preferable.

Keywords: **Super population model, bias, mean square error (*MSE*), efficiency.**

Introduction

To estimate the population mean, Chakrabarty [2] assumed a model for compare-son of estimators $\bar{Y}_{SR}$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ with $\bar{y}$ and $\hat{\bar{Y}}_R$. These estimators are given below:

(i) Mean estimator: $\bar{y}$

(ii) Mean ratio estimator: $\hat{\bar{Y}}_R = (\bar{y} / \bar{x})\bar{X}$,

(iii) Srivastava's estimator [10]:

$$\hat{\bar{Y}}_{SR} = \bar{y}(\bar{X} / \bar{x})^W,$$

(iv) Chakrabarty's estimator [2]: (a)

$$\hat{\bar{Y}}_{C_1} = (1-W)\bar{y} + W\hat{\bar{Y}}_R,$$

(v) Chakrabarty's estimator [2]: (b)

$$\hat{\bar{Y}}_{C_2} = (1-W)\bar{y} + Wt_Q\bar{X}$$

where $W\,(0 < W < 1)$ is fixed positive weight, $\bar{y}$ and $\bar{x}$ are the means of *yi* and *xi* observations respectively. $\bar{X}$ is assumed to be the known population mean of the auxiliary variable. Quenouille [6] introduced the estimator $t_Q = 2r - (r_1 + r_2)/2$ (where $r = \bar{y}/\bar{x}$ and $r_i = \bar{y}_i / \bar{x}_i$ (i = 1, 2) and has shown that the bias can be reduced from $O(n^{-1})$ to $O(n^{-2})$ by splitting the observations into two equal parts each of size n/2, for even n. Sahoo [8] suggested almost unbiased estimator $\hat{\bar{Y}}_S = r(1+\lambda c_{xy})(1-c_x^2)$, where $c_{xy} = s_{xy}/(\bar{x}.\bar{y})$, s_{xy} is the sample covariance, cx is the coefficient of variation of x and $\lambda = (\frac{1}{n} - \frac{1}{N})$. Dalabehera and Sahoo [4] discussed the efficiencies of six almost unbiased ratio estimators under the model $y_i = \beta x_i + u_i$ (i = 1, 2,....., n), whereβ is unknown real constant and u_i's are random errors. They have shown that $\hat{\bar{Y}}_S$ is more efficient than other estimators under certain conditions. In situation when regression line does not pass through origin then one cannot use this model. Instead we use the model as assumed by Singh [9], $y_i = \alpha + \beta x_i + u_i$, where α is constant. The u_i has the following properties.

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$E(u_i \mid x_i) = 0$

$E(u_i u_j \mid x_i, x_j) = 0 \quad i, j \quad (i \neq j)$

$Var(u_i \mid x_i) = n\delta$ (δ is the constant of $O(n^{-1})$).

We assume that x_i / n has a gamma distribution with parameter h, so that $\bar{x} = \sum x_i / n$ also has gamma distribution with parameter $m = nh$.

Chakrabarty [2] used this model to investigate exact bias and exact variance of estimators (iii), (iv) and (v). He concluded that the performance of $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ is better. Chakrabarty and Rao [3] used the same model to find the bias and variance of ratio estimator by using the jackknife technique. Durbin [5] used this model for comparison of Quenouille's estimator t_Q and ratio estimator $\hat{\bar{Y}}_R$. He observed that has smaller mean square error (*MSE*) than . Rao and Webster [7] used the same model to investigate the efficiencies of . Chakrabarty [1] also used this model to investigate the exact efficiency of and stability of the variance estimator of relative to that . He has shown that is superior to for $\rho \geq 0.4$ (ρ being the population correlation coefficient) in small samples and also the variance of the former is more stable.

Motivated by the Sahoo [8], we propose a ratio-type estimator which is a combination of $\bar{y}$ and $\hat{\bar{Y}}_S$ using constant weight W, as

$\hat{\bar{Y}}_J = (1-W)\bar{y} + W\hat{\bar{Y}}_S \bar{X}$.

A comparison of $\hat{\bar{Y}}_J$ is made with $\bar{y}$, $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ under Singh's [9] model with respect to bias and efficiency.

Exact Bias of Estimators

From the model, we have

$\bar{y} = \alpha + \beta\ \bar{x} + \bar{u}$, (1)

$E(\bar{y}) = \bar{Y} = \alpha + \beta\ m$,

$\hat{\bar{Y}}_R = (\alpha + \bar{u})m / \bar{x} + \beta\ m$

and

$r = (\alpha + \bar{u}) / \bar{x} + \beta$. (2)

We assume that $\bar{x}$ has a gamma distribution with density function $\bar{x}^{(m-1)}.e^{-\bar{x}} / \Big)m$ having mean m.

Using the above density function, we have

$E(1/\bar{x}) = 1/(m-1)$,

$E(1/\bar{x}^2) = 1/[(m-1)(m-2)]$.

When sample is divided into two equal halves, the sample means $\bar{x}_1$ and $\bar{x}_2$ of two halves have independent gamma distributions each with parameter m/2. Therefore, from Durbin [5], we have

$E(1/\bar{x}_i) = 1/(m-2)$,

$E(1/\bar{x}_i^2) = 1/(m-2)(m-4)$, (i = 1, 2).

Using above results, biases of various estimators are given in the following section.

Biases

(i) For classical ratio estimator $\hat{\bar{Y}}_R$

$Bias(\hat{\bar{Y}}_R) = Bias(r)\bar{X}$. (3)

By (2) and (3), it can be easy to show that

$Bias(\hat{\bar{Y}}_R) = \alpha/(m-1)$. (4)

(ii) For Chakrabarty's estimators $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$

$Bias(\hat{\bar{Y}}_{C_1}) = WBias(\hat{\bar{Y}}_R)$, (5)

$Bias(\hat{\bar{Y}}_{C_2}) = WBias(t_Q)\bar{X}$, (6)

Table1. **Efficiencies for selected values of *m*, ρ and *K* with $W = 0.25$.**

m	*K*	$\rho = 0.2$				$\rho = 0.5$				$\rho = 0.8$			
		E_r	E_{c_1}	E_{c_2}	E_J	E_r	E_{c_1}	E_{c_2}	E_J	E_r	E_{c_1}	E_{c_2}	E_J
	0.25	68	94	100	100	79	98	104	104	89	102	107	109
	0.50	61	95	100	101	87	105	110	111	139	116	121	121*
8	0.75	49	95	99	101	79	110	115	116	181	130	135	135*
	1.00	37	93	96	100	62	114	117	120	160	145	148	149*
	1.25	28	90	91	98	45	116	116	123	107	161	159	165
	0.25	85	98	101	102	100	103	105	106	117	107	110	110*
	0.50	77	100	103	103	109	109	112	112	178	119	122	122*
16	0.75	63	100	103	103	100	114	117	117	226	133	136	136*
	1.00	49	99	101	102	80	119	121	122	203	148	150	150*
	1.25	37	97	99	101	59	122	124	126	140	165	165	166
	0.25	91	100	102	102	107	104	106	106*	128	108	110	110*
	0.50	83	101	103	103	117	110	112	112*	192	120	122	122*
24	0.75	68	101	103	104	107	116	117	118	242	134	136	136*
	1.00	53	100	102	103	86	120	122	122	218	149	150	151*
	1.25	41	99	101	102	64	124	125	126	152	166	166	167
	0.25	94	100	102	102	111	104	106	106*	133	109	110	110*
	0.50	86	102	103	103	121	111	112	112*	199	121	122	122*
32	0.75	71	102	103	104	111	116	117	118	250	135	136	136*
	1.00	55	102	103	103	89	121	122	123	225	150	151	151*
	1.25	43	100	101	102	67	125	126	127	158	166	166	167

Table 2. **Efficiencies for selected values of *m*, ρ and *K* with $W = 0.50$.**

m	*K*	$\rho = 0.2$				$\rho = 0.5$				$\rho = 0.8$			
		E_r	E_{c_1}	E_{c_2}	E_J	E_r	E_{c_1}	E_{c_2}	E_J	E_r	E_{c_1}	E_{c_2}	E_J
	0.25	68	86	91	98	79	94	99	106				
	0.50	61	85	89	97	87	104	109	117	89	101	104	115
8	0.75	49	80	81	93	79	108	112	122	139	129	135	144
	1.00	37	73	71	85	62	106	105	122	181	162	169	178*
	1.25	28	64	60	76	45	98	93	115	160	192	193	211
										107	207	192	232
	0.25	85	95	100	101	100	103	108	110	117	112	117	119
	0.50	77	95	99	101	109	114	119	121	178	141	146	148*
16	0.75	63	91	95	98	100	120	125	127	226	174	180	182*
	1.00	49	84	87	91	80	120	124	129	202	208	213	218
	1.25	37	76	78	83	59	115	118	124	140	233	235	246
	0.25	91	98	101	102	107	106	110	111	127	116	119	121*
	0.50	83	98	100	102	117	117	120	122	192	144	148	149*
24	0.75	68	94	97	99	107	124	127	129	242	178	182	183*
	1.00	53	88	90	93	86	125	128	130	218	213	217	219
	1.25	41	80	82	85	65	120	123	126	151	241	244	250
	0.25	90	97	99	100	111	108	110	111	133	118	120	121*
	0.50	78	94	97	98	121	119	121	122	199	146	149	150*
32	0.75	63	89	91	92	111	125	128	129	250	180	183	184*
	1.00	49	82	84	85	89	127	129	131	226	216	218	220*
	1.25	38	73	75	77	67	123	125	128	158	245	247	251

Table 3. **Biases for selected values of *m*, ρ and *K* with $W = 0.25$.**

m	K	$\rho = 0.2$				$\rho = 0.5$				$\rho = 0.8$			
		B_r	B_{c_1}	B_{c_2}	B_J	B_r	B_{c_1}	B_{c_2}	B_J	B_r	B_{c_1}	B_{c_2}	B_J
	0.25	1.67	0.49	0.17	0.06	8.99	2.50	0.86	0.29	20.95	5.62	1.92	0.64
	0.50	9.48	2.96	1.01	0.34	0.00	0.00	0.00	0.00	14.29	3.26	1.11	0.37
8	0.75	15.56	5.41	1.84	0.62	8.99	2.65	0.90	0.30	2.72	0.58	0.20	0.07
	1.00	19.74	7.80	2.64	0.90	15.88	5.39	1.82	0.62	10.22	2.44	0.82	0.27
	1.25	22.48	10.08	3.38	1.17	20.37	8.16	2.73	0.93	18.81	5.77	1.91	0.65
	0.25	1.23	0.33	0.05	0.02	6.67	1.69	0.24	0.10	15.87	3.79	0.55	0.23
	0.50	7.03	2.00	0.29	0.12	0.00	0.00	0.00	0.00	10.67	2.19	0.32	0.13
16	0.75	11.65	3.66	0.53	0.22	6.67	1.78	0.26	0.11	2.00	0.38	0.06	0.02
	1.00	14.91	5.31	0.77	0.32	11.89	3.63	0.52	0.22	7.59	1.62	0.23	0.10
	1.25	17.10	6.90	1.00	0.41	15.40	5.53	0.80	0.33	14.18	3.85	0.55	0.23
	0.25	1.02	0.27	0.02	0.01	5.52	1.36	0.12	0.05	13.24	3.05	0.28	0.12
	0.50	5.82	1.61	0.15	0.06	0.00	0.00	0.00	0.00	8.85	1.75	0.16	0.07
24	0.75	9.68	2.95	0.27	0.12	5.52	1.43	0.13	0.06	1.66	0.31	0.03	0.01
	1.00	12.42	4.27	0.39	0.17	9.88	2.92	0.27	0.12	6.29	1.30	0.12	0.05
	1.25	14.28	5.57	0.59	0.23	12.84	4.45	0.41	0.18	11.80	3.08	0.28	0.12
	0.25	0.89	0.23	0.02	0.01	4.81	1.17	0.08	0.04	11.59	2.62	0.18	0.08
	0.50	5.08	1.38	0.09	0.04	0.00	0.00	0.00	0.00	7.73	1.51	0.10	0.05
32	0.75	8.45	2.53	0.17	0.08	4.81	1.23	0.08	0.04	1.44	0.26	0.02	0.01
	1.00	10.86	3.68	0.25	0.11	8.63	2.51	0.17	0.08	5.48	1.12	0.07	0.03
	1.25	12.51	4.79	0.32	0.15	11.24	3.82	0.26	0.12	10.32	2.65	0.18	0.08

Table 4. **Biases for selected values of *m*, ρ and *k* with $W = 0.50$.**

m	K	$\rho = 0.2$				$\rho = 0.5$				$\rho = 0.8$			
		B_r	B_{c_1}	B_{c_2}	B_J	B_r	B_{c_1}	B_{c_2}	B_J	B_r	B_{c_1}	B_{c_2}	B_J
	0.25	1.67	0.94	0.32	0.11	8.99	4.89	1.67	0.58	20.95	11.18	3.78	1.32
	0.50	9.48	5.60	1.90	0.66	0.00	0.00	0.00	0.00	14.29	6.89	2.35	0.81
8	0.75	15.56	9.96	3.34	1.19	8.99	5.26	1.78	0.62	2.72	1.29	0.44	0.15
	1.00	19.74	13.77	4.53	1.66	15.88	10.42	3.46	1.24	10.22	5.60	1.87	0.65
	1.25	22.48	16.93	5.47	2.06	20.37	15.02	4.87	1.81	18.81	13.07	4.20	1.54
	0.25	1.23	0.65	0.10	0.04	6.67	3.39	0.50	0.21	15.87	7.77	1.13	0.47
	0.50	7.03	3.90	0.57	0.24	0.00	0.00	0.00	0.00	10.67	4.75	0.69	0.29
16	0.75	11.65	6.99	1.02	0.43	6.67	3.65	0.53	0.22	2.00	0.88	0.13	0.05
	1.00	14.91	9.78	1.42	0.60	11.89	7.31	1.06	0.44	7.59	3.85	0.56	0.23
	1.25	17.10	12.18	1.77	0.75	15.40	10.71	1.55	0.66	14.18	9.16	1.31	0.55
	0.25	1.02	0.53	0.05	0.02	5.52	2.75	0.25	0.11	13.24	6.30	0.58	0.26
	0.50	5.82	3.16	0.29	0.13	0.00	0.00	0.00	0.00	8.85	3.84	0.35	0.16
24	0.75	9.68	5.69	0.52	0.23	5.52	2.96	0.27	0.12	1.66	0.71	0.07	0.03
	1.00	12.42	7.99	0.74	0.33	9.88	5.95	0.55	0.24	6.29	3.11	0.29	0.13
	1.25	14.28	9.98	0.92	0.41	12.84	8.75	0.80	0.36	11.80	7.44	0.68	0.30
	0.25	0.89	0.45	0.03	0.01	4.81	2.37	0.16	0.07	11.59	5.44	0.37	0.17
	0.50	5.08	2.73	0.18	0.08	0.00	0.00	0.00	0.00	7.73	3.31	0.22	0.10
32	0.75	8.45	4.92	0.33	0.15	4.81	2.55	0.17	0.08	1.44	0.61	0.04	0.02
	1.00	10.86	6.91	0.47	0.21	8.63	5.14	0.35	0.16	5.48	2.68	0.18	0.08
	1.25	12.51	8.66	0.58	0.27	11.24	7.58	0.51	0.23	10.32	6.43	0.43	0.20

therefore using (4) and (5), we have

$$Bias(\hat{\bar{Y}}_{C_1}) = W\alpha/(m-1). \quad (7)$$

To find $Bias(t_Q)$, split the sample into two halves, having the ratio estimators r_1 and r_2. Under the model, if we assume $\bar{y}_1 = \alpha + \beta.\bar{x}_1 + \bar{u}_1$, $\bar{y}_2 = \alpha + \beta.\bar{x}_2 + \bar{u}_2$ and $\bar{u} = (\bar{u}_1 + \bar{u}_2)/2$, then $Bias(t_Q)$ can be shown to be

$$Bias(t_Q) = -2\alpha/[m(m-1)(m-2)]. \quad (8)$$

Using (6) and (8), we get

$$Bias(\hat{\bar{Y}}_{C_2}) = -2W\alpha/(m-1)(m-2). \quad (9)$$

(iii) For modified estimator $\hat{\bar{Y}}_J$

$$Bias(\hat{\bar{Y}}_J) = WBias(\hat{\bar{Y}}_S)\bar{X}, \quad (10)$$

where

$$Bias(\hat{\bar{Y}}_S) = \alpha/[m(m-1)(m+1)], \quad (11)$$

therefore using (10) and (11), we have

$$Bias(\hat{\bar{Y}}_J) = W\alpha/[(m-1)(m+1)]. \quad (12)$$

Comparison of Exact Biases

Note that

(i) $|Bias(\hat{\bar{Y}}_J)| < |Bias(\hat{\bar{Y}}_R)|$ if $W < (m+1)$,

(ii) $|Bias(\hat{\bar{Y}}_J)| < |Bias(\hat{\bar{Y}}_{C_1})|$ if $m > 0$,

(iii) $|Bias(\hat{\bar{Y}}_J)| < |Bias(\hat{\bar{Y}}_{C_2})|$ if $(m+4) > 0$.

Since we assume that $m > 0$ in Tables 1 through 4 and $(0 < W < 1)$, therefore $|Bias(\hat{\bar{Y}}_J)|$ will always be less than $|Bias(\hat{\bar{Y}}_R)|$, $|Bias(\hat{\bar{Y}}_{C_1})|$ and $|Bias(\bar{Y}_{C_2})|$.

For special case the estimators $\hat{\bar{Y}}_{C_1}$, $\hat{\bar{Y}}_{C_2}$ and $\hat{\bar{Y}}_J$ become unbiased for $\alpha = 0$ (i.e. regression line passes through origin).

Exact MSEs

Chakrabarty [2] used a method similar to Rao and Webster [7] to obtain variances of $\bar{y}$, $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$. Using the same approach we obtain the MSE of these estimators which are

$$MSE(\bar{y}) = \beta^2 m + \delta,$$

$$MSE(\hat{\bar{Y}}_R) = \alpha^2 A_1 + \delta A_2,$$

$$MSE(\hat{\bar{Y}}_{C_1}) = \alpha^2 W^2 B_1 + \beta^2 m(1-W)^2 - 2\alpha\beta W(1-W)B_3 + \delta[W^2 A_2 + W(1-W)B_2 + (1-W)],$$

$$MSE(\hat{\bar{Y}}_{C_2}) = \alpha^2 W^2 D_1 + \beta^2 m(1-W)^2 + \delta[(1-W)^2 + W^2 D_2 + 2W(1-W)D_3] - 2\alpha\beta W(1-W)D_3,$$

Where $A_1 = \dfrac{(m^2+m-2)}{(m-1)^2(m-2)}$,

$$A_2 = \frac{m^2}{(m-1)(m-2)}, \; B_1 = \frac{(m+2)}{(m-1)(m-2)},$$

$$B_2 = \frac{(m+1)}{(m-1)}, \; B_3 = \frac{m}{(m-1)},$$

$$D_1 = \frac{m^3 - 5m^2 + 12m + 16}{(m-1)(m-2)^2(m-4)},$$

$$D_2 = \frac{(m^2 - 7m + 18)m^2}{(m-1)(m-2)^2(m-4)}, \text{ and}$$

$$D_3 = \frac{m(m-3)}{(m-1)(m-2)}.$$

To obtain the MSE of $\hat{\bar{Y}}_J$, Theorem 3 of Rao and Webster [7], reproduced as under has been used.

Theorem

Let $Z_1, ..., Z_n$ be independent gamma variates with parameter h, where $Z_i = x_i / n$. Then, for $i \neq j$,

$$E\left\{\frac{Z_i^a Z_j^b}{(\sum Z_t)^c}\right\} = \frac{\overline{(a+h).}\overline{(b+h)}}{(\overline{h})^2}\cdot\frac{1}{\prod_{t=1}^{c}(m+a+b-t)},$$

where c is an integer greater than zero and a and b are integers greater than or equal to zero.

Using the above theorem, the MSE of $\hat{\bar{Y}}_J$ is

$$MSE(\hat{\bar{Y}}_J) = \alpha^2 W^2(E_1+E_2) + \delta[(1-W)^2 + 2W(1-W)E_3 + W^2(E_2+E_4)] + \beta^2 m(1-W)^2 - 2\alpha\beta W(1-W)E_3,$$

where $E_1 = \frac{(m^2-m+2)}{(m-1)(m+1)(m-2)}$,

$$E_2 = \frac{m^2[6(n-1)+m(n+1)]}{(n-1)(m-1)(m-2)(m+1)(m+2)(m+3)},$$

$$E_3 = \frac{m^2}{(m-1)(m+1)} \text{ and } E_4 = \frac{m^2}{(m-2)(m+1)}.$$

Comparison of *MSE*s and Results

We use the MSE criteria for comparison of estimators $\bar{y}$, $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$, $\hat{\bar{Y}}_{C_2}$ with $\hat{\bar{Y}}_J$ under the following conditions.

(i) $MSE(\hat{\bar{Y}}_J) < MSE(\bar{y})$ if
$\beta^2 mW(2-W) - \alpha^2 W^2(E_1+E_2) + 2\alpha\beta W(1-W)E_3 + \delta W[2-W-2(1-W)E_3 - W(E_2+E_4)] > 0$.

(ii) $MSE(\hat{\bar{Y}}_J) < MSE(\hat{\bar{Y}}_R)$ if
$\alpha^2[A_1 - W^2(E_1+E_2)] - \beta^2 m(1-W)^2 + 2\alpha\beta W(1-W)E_3 + \delta[A_2 - (1-W)^2 - 2W(1-W)E_3 - W^2(E_2+E_4)] > 0$.

(iii) $MSE(\hat{\bar{Y}}_J) < MSE(\hat{\bar{Y}}_{C_1})$ if
$\alpha^2 W^2(B_1 - E_1 - E_2) + \delta W[W(A_2 - E_2 - E_4) + (1-W)(1+B_2-2E_3)] + 2\alpha\beta W(1-W)(E_3 - B_2) > 0$.

(iv) $MSE(\hat{\bar{Y}}_J) < MSE(\hat{\bar{Y}}_{C_2})$ if
$\alpha^2 W^2(D_1 - E_1 - E_2) + \delta W[W(D_2 - E_2 - E_4) + 2(1-W)(D_3 - E_3)] + 2\alpha\beta W(1-W)(E_3 - D_3) > 0$.

Results and Discussion

From Chakrabarty and Rao [3] and Chakrabarty ([1,2], we have

$\alpha = \bar{Y}[(K-\rho)K^{-1}]$,

$\beta = \bar{Y}[\rho(Km)^{-1}]$ and

$\delta = \bar{Y}^2[(1-\rho^2)(K^2 m)^{-1}]$, where $K = c_x / c_y$.

The exact efficiencies of $\hat{\bar{Y}}_R$ and $\hat{\bar{Y}}_i$ $(i = c_1, c_2, \text{J})$ relative to $\bar{y}$ are defined as

$E_r = [MSE(\bar{y}) / MSE(\hat{\bar{Y}}_R)]\times 100$,

$E_i = [MSE(\bar{y}) / MSE(\hat{\bar{Y}}_i)]\times 100$, , J).

After substituting values of a, b and d; E_r and E_i can be expressed as a function of K, m, r and W. The results are arranged in Tables 1 and 2 for various values of K, m, r at $W = 0.25$ and 0.50. In these Tables, it can be observed that efficiency of all estimators increases as r and K increase. The estimator $\hat{\bar{Y}}_J$ is generally more efficient than $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ except the asterisk '*'cases where $\hat{\bar{Y}}_R$ is superior (Tables 1 and 2). From our model, $c_x = h^{-1/2}$, $c_{\bar{x}} = m^{-1/2}$ and n £ m when h ³1 then it may be concluded that $W = 0.25$ and 0.50 is a good choice for the estimators $\hat{\bar{Y}}_{C_1}$, $\hat{\bar{Y}}_{C_2}$ and $\hat{\bar{Y}}_J$. In analysis we use $n = m$ for our convenience.

To find the suitability of best estimator, we look at the biases in addition to MSEs.

The absolute biases of estimators $\hat{\bar{Y}}_R$ and $\hat{\bar{Y}}_i$ $(i = c_1, c_2, J)$ relative to the *MSE*s are defined as:

$B_R = |B(\hat{\bar{Y}}_R)| \times 100 / [MSE(\hat{\bar{Y}}_R)]^{1/2}$,

$B_i = |B(\bar{y}_i)| \times 100 / [MSE(\bar{y}_i)]^{1/2}$ $(i = c_1, c_2, J)$.

From Tables 3 and 4, the maximum biases for estimators $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ and $\hat{\bar{Y}}_J$ are around 22%, 17%, 5% and 1% respectively. The bias of $\hat{\bar{Y}}_R$ is the largest for K ≥ 1. Bias of $\hat{\bar{Y}}_{C_2}$ < 1% for all values of ρ, K and m ≥16. Bias of $\hat{\bar{Y}}_R$ is considerably higher than all other biases of estimators. The bias of $\hat{\bar{Y}}_J$ is much smaller than the biases of $\hat{\bar{Y}}_R$, $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$ So $\hat{\bar{Y}}_J$ is preferable where freedom from bias is required.

Conclusion

Four estimators are compared with respect to bias and efficiency under the super population model. The modified estimator $\hat{\bar{Y}}_J$ is more efficient than $\hat{\bar{Y}}_R$ (except the asterisk '*' values) and is comparable with $\hat{\bar{Y}}_{C_1}$ and $\hat{\bar{Y}}_{C_2}$. Although we have calculated efficiencies and biases by selecting only two values of W i.e. 0.25 and 0.50, these cover the interesting range of weights. It is also interesting to note that at $\rho = K$, all the estimators become unbiased. The efficiencies tend to increase as values of ρ, K and m increases. The gain in efficiency is higher for $\rho = 0.8$ and $m \geq 24$ in Tables 1 and 2. In Table 3, the bias is much smaller for $\rho = 0.8$ and for (i) $m = 8$, 16 and $K = 0.75$; (ii) $m = 24$ and $0.50 \leq K \leq 0.1$; (iii) $m = 32$ and $0.25 \leq K \leq 1.25$. In Table 4, reduction in bias is for $\rho = 0.8$ and for the following values: (i) $m = 16$, 24 and $K = 0.75$; (ii) $m = 32$ and $0.75 \leq K \leq 1$; (iii) $m = 32$ and $0.25 \leq K \leq 1.25$.

It is observed that $\hat{\bar{Y}}_{C_2}$ is also more efficient as compared to $\hat{\bar{Y}}_R$ and $\hat{\bar{Y}}_{C_1}$ but in practice can create a problem because of splitting samples into two equal parts under the estimator t_Q. So $\hat{\bar{Y}}_J$ is preferable for estimating the population parameters.

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CONSEQUENCES OF MIXED NUMBER LORENTZ TRANSFORMATION

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Abstract: In terms of mixed number we can derive the Lorentz transformation, which we call the Mixed number Lorentz Transformations. The mixed number Lorentz transformation is better than the Lorentz transformation. In this paper we have discussed some consequences of mixed number Lorentz transformation such as length contraction, time dilation and relativistic velocity addition formula of mixed number Lorentz transformation.

***Keywords*:** Mixed number Lorentz transformation, length contraction and time dilation.

Introduction

Let us consider two inertial frames of reference S and S′, where the frame S is at rest and the frame S′ is moving along X-axis with velocity **V** with respect to S frame. The space and time coordinates of S and S′ are (x, y, z, t) and (x′, y′, z′, t′), respectively. The relation between the coordinates of S and S′, which is called special Lorentz transformation, can be written [1] as

$$\left.\begin{aligned} x' &= \frac{x - vt}{\sqrt{1 - v^2/c^2}} \\ y' &= y \\ z' &= z \\ t' &= \frac{t - vx/c^2}{\sqrt{1 - v^2/c^2}} \end{aligned}\right| \qquad (1)$$

and

$$\left.\begin{aligned} x &= \frac{x' + vt'}{\sqrt{1 - v^2/c^2}} \\ y &= y' \\ z &= z' \\ t &= \frac{t' + (v/c^2)x'}{\sqrt{1 - v^2/c^2}} \end{aligned}\right| \qquad (2)$$

When the velocity **V** of S′ with respect to the S is not along X-axis i.e. the velocity **V** has three components V_x, V_y and V_z then the relation between the coordinates of S and S′, which is called most general Lorentz transformation, can be written [2] as

$$\begin{aligned} \mathbf{X}' = \quad & \mathbf{X} + \mathbf{V}\,[\{(\mathbf{X}.\mathbf{V})/v^2\}\{(1 - v^2/c^2)^{-1/2} - 1\} \\ & - t(1 - v^2/c^2)^{-1/2}] \\ t' = \quad & (1 - v^2/c^2)^{-1/2}\,(t - \mathbf{V}.\mathbf{X}/c^2) \end{aligned} \qquad (3)$$

From these transformation equations, addition of velocities can be written as

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$$\mathbf{U}' = \frac{\mathbf{X}'}{t'} = \frac{\mathbf{X} + \mathbf{V}[\{(\mathbf{X}.\mathbf{V})/v^2\}\{\gamma - 1\} - t\gamma]}{\gamma(t - \mathbf{V}.\mathbf{X}/c^2)} \quad (4)$$

where $\gamma = \frac{1}{\sqrt{1 - v^2/c^2}}$

Dividing the numerator and the denominator of equation (4) by t, we get

$$\mathbf{U}' = \frac{\mathbf{X}'}{t'} = \frac{\mathbf{U} + \mathbf{V}[\{(\mathbf{U}.\mathbf{V})/v^2\}\{\gamma - 1\} - \gamma]}{\gamma(1 - \mathbf{V}.\mathbf{U}/c^2)} = \mathbf{V} \oplus \mathbf{U} \quad (5)$$

where $\mathbf{U} = \mathbf{X}/t$ and the symbol $\oplus$ denotes the Lorentz sum of velocities.

The most general Lorentz transformation has some limitations [3] such as

(i) It is non-associative.
(ii) The space generated by the most general Lorentz transformation is not isotropic (in the sense that the triangular law of addition of velocities is valid for velocities in some directions and not valid for velocities in other directions).
(iii) It does not have group property without rotation.

To overcome these limitations of most general lorentz transformations we have derived the mixed number Lorentz transformation [3], which is as follows

Using c = 1 and $\gamma = \frac{1}{\sqrt{1 - v^2/c^2}}$ in equation (1) and (2), we can write

$$x' = \gamma(x - vt) \quad 6(i)$$

$$t' = \gamma(t - vx) \quad 6(ii)$$

$$x = \gamma(x' + vt') \quad 7(i)$$

$$t = \gamma(t' + vx') \quad 7(ii)$$

Adding equations 6(i) and 6(ii), we get

$$t' + x' = \gamma(t - vx + x - vt)$$
$$t' + x' = \gamma\{(t + x) - v(t + x)\} \quad (8)$$

Using $(t' + x') = p'$ and $(t + x) = p$ in equation (8), we can write

$$p' = \gamma(p - pv) \quad (9)$$

Adding equations 7(i) and 7(ii), we get

$$t + x = \gamma(t' + vx' + x' + vt')$$
$$t + x = \gamma\{(t' + x') + v(t' + x')\} \quad (10)$$

Using $(t' + x') = p'$ and $(t + x) = p$ in equation (10), we can write

$$p = \gamma(p' + p'v) \quad (11)$$

In the case of most general Lorentz transformation, the velocity **V** of S′ with respect to the S is not along X-axis i.e. the velocity **V** has three components V_x, V_y and V_z. In this case, **Z** and **Z′** is the space part in S and S′ frame, respectively, and hence equation (9) can be written as

$$P' = \gamma(P - P\mathbf{V})$$

where $P' = (t' + \mathbf{Z}')$, and $P = (t + \mathbf{Z})$ are two mixed numbers [4,5] like quaternions [6,7,8].

$$\therefore (t' + \mathbf{Z}') = \gamma\{(t + \mathbf{Z}) - (t + \mathbf{Z})\mathbf{V}\}$$
$$\text{or, } (t' + \mathbf{Z}') = \gamma\{(t + \mathbf{Z}) - (t + \mathbf{Z})(0 + \mathbf{V})\} \quad (12)$$

The product of two mixed numbers $\alpha = (x + \mathbf{A})$ and $\beta = (y + \mathbf{B})$ can be written [4,9,10] as

$$(x + \mathbf{A})(y + \mathbf{B}) = xy + \mathbf{A}.\mathbf{B} + x\mathbf{B} + y\mathbf{A} + i\mathbf{A}\times\mathbf{B} \quad (13)$$

Using equation (13), we can write

$$(t + \mathbf{Z})(0 + \mathbf{V}) = \mathbf{Z}.\mathbf{V} + t\mathbf{V} + i\mathbf{Z}\times\mathbf{V} \quad (14)$$

From equations (12) and (13), we get

$$(t' + \mathbf{Z}') = \gamma\{t + \mathbf{Z} - (\mathbf{Z}.\mathbf{V} + t\mathbf{V} + i\mathbf{Z}\times\mathbf{V})\}$$
$$\text{or, } (t' + \mathbf{Z}') = \gamma(t - \mathbf{Z}.\mathbf{V}) + \gamma(\mathbf{Z} - t\mathbf{V} - i\mathbf{Z}\times\mathbf{V}) \quad (15)$$

According to mixed number algebra, we can write from equation (15)

$$\left.\begin{aligned} t' &= \gamma\,(t - \mathbf{Z}.\mathbf{V}) \text{ and} \\ \mathbf{Z}' &= \gamma\,(\mathbf{Z} - t\mathbf{V} - i\mathbf{Z}\times\mathbf{V}) \end{aligned}\right| \qquad (16)$$

Similarly, we can show that

$$\left.\begin{aligned} t &= \gamma\,(t' + \mathbf{Z}'.\mathbf{V}) \text{ and} \\ \mathbf{Z} &= \gamma\,(\mathbf{Z}' + t'\mathbf{V} + i\mathbf{Z}'\times\mathbf{V}) \end{aligned}\right| \qquad (17)$$

Equations (16) and (17) are the mixed number Lotentz transformation [3].

From equation (17), dividing **Z** by t, we get

$$\frac{\mathbf{Z}}{t} = \frac{(\mathbf{Z}' + t'\mathbf{V} + i\mathbf{Z}'\times\mathbf{V})}{(t' + \mathbf{Z}'.\mathbf{V})} \qquad (18)$$

Dividing the numerator and the denominator by t′, we get

$$\frac{\mathbf{Z}}{t} = \frac{\{(\mathbf{Z}'/t') + \mathbf{V} + i(\mathbf{Z}'/t')\times\mathbf{V}\}}{\{1 + (\mathbf{Z}'/t').\mathbf{V}\}}$$

$$= \frac{\mathbf{U} + \mathbf{V} + i\,\mathbf{U}\times\mathbf{V}}{1 + \mathbf{U}.\mathbf{V}} \quad (\text{where } \mathbf{U} = \mathbf{Z}'/t')$$

$$\text{or, } \mathbf{W} = \mathbf{V} \oplus \mathbf{U} = \frac{\mathbf{U} + \mathbf{V} + i\,\mathbf{U}\times\mathbf{V}}{1 + \mathbf{U}.\mathbf{V}} \qquad (19)$$

where $\mathbf{W} = \mathbf{Z}/t$

Equation (19) is the Lorentz sum of velocities for the mixed number Lorentz transformation.

We now discuss the following consequences of mixed number Lorentz transformation.

Length Contraction

(i) *Length contraction of special Lorentz transformation*

The length of any object in a moving frame will appear foreshortened (or contracted) in the direction of motion. The amount of contraction can be calculated from the Lorentz transformation. The length is maximum in the frame in which the object is at rest.

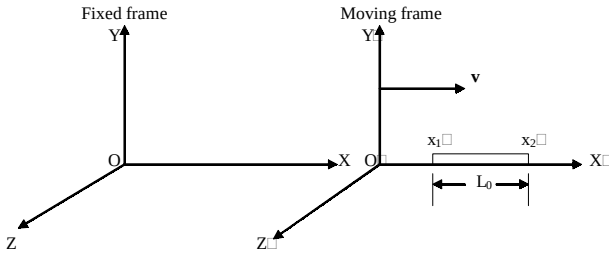

Figure 1

If the length $L_0 = x_2' - x_1'$ is measured in the moving reference frame, then $L = x_2 - x_1$ can be calculated using the special Lorentz transformation (equation 1).

$$L_0 = x_2' - x_1' = L\gamma$$
$$L_0 = L\gamma \qquad (20)$$

This is the formula of length contraction of special Lorentz transformation.

where $\gamma = \frac{1}{\sqrt{1 - v^2/c^2}}$

(ii) *Length contraction of the most general Lorentz transformation*

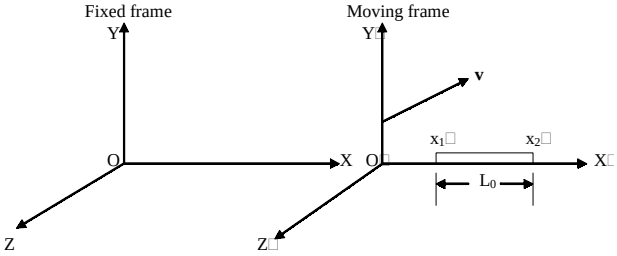

Figure 2

$\mathbf{L}_0 = \mathbf{x}_2' - \mathbf{x}_1'$

Using equation (3), we can write

$\mathbf{x}_2' = \mathbf{x}_2 + \mathbf{v}[\{(\mathbf{x}_2.\mathbf{v})/v^2\}\{(\gamma - 1) - t\gamma\}]$

and $\mathbf{x}_1' = \mathbf{x}_1 + \mathbf{v}[\{(\mathbf{x}_1.\mathbf{v})/v^2\}\{(\gamma - 1) - t\gamma\}]$

where $\gamma = \dfrac{1}{\sqrt{1 - v^2/c^2}}$

or, $\mathbf{L}_0 = \mathbf{x}_2 + \mathbf{v}[\{(\mathbf{x}_2.\mathbf{v})/v^2\}\{(\gamma - 1) - t\gamma\}] - \mathbf{x}_1 - \mathbf{v}[\{(\mathbf{x}_1.\mathbf{v})/v^2\}\{(\gamma - 1) - t\gamma\}]$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}[\{(\mathbf{x}_2.\mathbf{v})/v^2\}\{(\gamma - 1) - t\gamma] - \mathbf{v}[\{(\mathbf{x}_1.\mathbf{v})/v^2\}\{(- 1) - t\gamma]$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}\{(\mathbf{x}_2.\mathbf{v})/v^2\}\{(\gamma - 1)\} - t\gamma\mathbf{v} - \mathbf{v}\{(\mathbf{x}_1.\mathbf{v})/v^2\}\{(\gamma - 1)\} + t\gamma\mathbf{v}$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}\{(\mathbf{x}_2.\mathbf{v})/v^2 - (\mathbf{x}_1.\mathbf{v})/v^2\}\{(\gamma - 1)\}$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}[\{(\mathbf{x}_2.\mathbf{v}) - (\mathbf{x}_1.\mathbf{v})\}/v^2\}](\gamma - 1)$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}[\{(x_2 v\cos\theta - x_1 v\cos\theta)\}/v^2\}](\gamma - 1)$

$= \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}[\{(x_2\cos\theta - x_1\cos\theta)\}/v\}](\gamma - 1)$

or, $\mathbf{L}_0 = \mathbf{x}_2 - \mathbf{x}_1 + \mathbf{v}[\{(x_2\cos\theta - x_1\cos\theta)\}/v\}](\gamma - 1)$ (21)

This is the formula of length contraction of the most general Lorentz transformation. If **v** is along x – axis, then $\theta = 0°$. We get from equation (21)

$\therefore L_0 = x_2 - x_1 + v[\{(x_2 - x_1)\}/v\}](\gamma - 1)$

or, $L_0 = x_2 - x_1 + (x_2 - x_1)(\gamma - 1)$

or, $L_0 = \gamma(x_2 - x_1) = \gamma L$

or, $L_0 = \gamma L$

which is exactly the same as equation (20).

(iii) *Length contraction of mixed number Lorentz transformation*

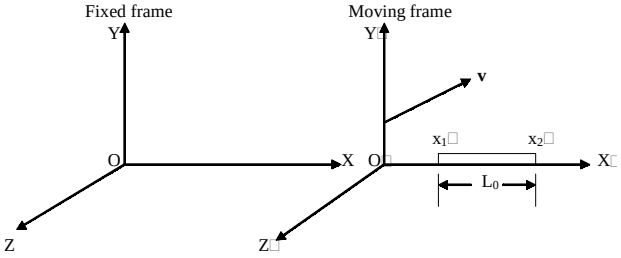

Figure 3

$L_0 = x_2' - x_1'$

Using equation (16), we can write

$\mathbf{x}_2' = \gamma(\mathbf{x}_2 - t\mathbf{v} - i\mathbf{x}_2\times\mathbf{v})$

and $\mathbf{x}_1' = \gamma(\mathbf{x}_1 - t\mathbf{v} - i\mathbf{x}_1\times\mathbf{v})$

where $\gamma = \dfrac{1}{\sqrt{1 - v^2/c^2}}$

or, $L_0 = \gamma(\mathbf{x}_2 - t\mathbf{v} - i\mathbf{x}_2\times\mathbf{v}) - \gamma(\mathbf{x}_1 - t\mathbf{v} - i\mathbf{x}_1\times\mathbf{v})$

$= \gamma(\mathbf{x}_2 - t\mathbf{v} - i\mathbf{x}_2\times\mathbf{v} - \mathbf{x}_1 + t\mathbf{v} + i\mathbf{x}_1\times\mathbf{v})$

$= \gamma\{\mathbf{x}_2 - \mathbf{x}_1 - i(\mathbf{x}_2 - \mathbf{x}_1)\times\mathbf{v}\}$

or, $L_0 = \gamma(\mathbf{L} - i\mathbf{L}\times\mathbf{v})$ (22)

This is the formula of length contraction of mixed number Lorentz transformation. If **v** is along x – axis, then $\mathbf{L}\times\mathbf{v} = 0$. We get from equation (22)

$L_0 = \gamma L$, which is exactly the same as equation (20).

Time dilation

(i) *Time dilation of special Lorentz Transformation*

A clock in a moving frame will be seen to be running slow or "dilated" according to Lorentz

transformation. The time will always be shortest as measured in its rest frame. The time measured in the frame in which the clock is at rest is called the "proper time".

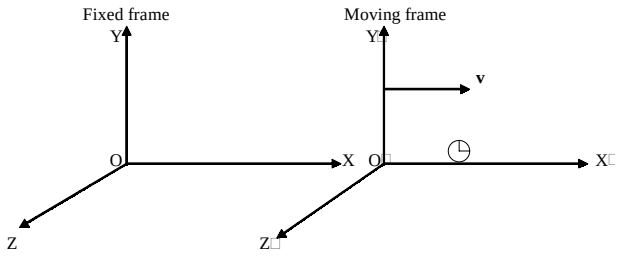

Figure 4

If the time interval $T_0 = t_2' - t_1'$ is measured in the moving reference frame, then $T = t_2 - t_1$ can be calculated using the special Lorentz transformation (equation 1).

$$T = t_2 - t_1 = \gamma \left[t_2' + \frac{vx_2'}{c^2} - t_1' + \frac{vx_1'}{c^2} \right]$$

The time measurements can be made in the moving frame at the same location, so the expression reduces to

$$T = \gamma \ (t_2' - t_1')$$

or, $T = T_0\gamma$
(23)

This is the formula of time dilation of special Lorentz transformation.

(ii) *Time dilation of most general Lorentz transformation*

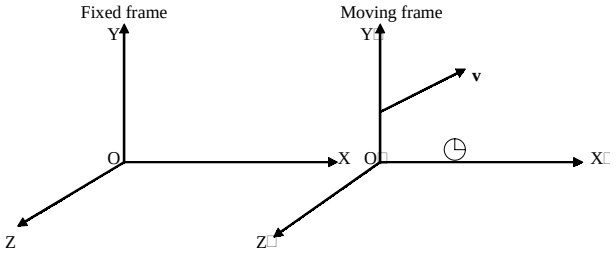

Figure 5

$T = t_2 - t_1$ can be calculated using the most general Lorentz transformation (equation 3).

$$T = t_2 - t_1 = \gamma (t_2' + \mathbf{V.X}'/c^2) - \gamma(t_1' + \mathbf{V.X}'/c^2)$$

or, $T = \gamma (t_2' - t_1') = \gamma T_0$

or, $T = \gamma T_0$

This is the formula of time dilation of most general Lorentz transformation, which is the same as that of special Lorentz transformation.

(iii) *Time dilation of mixed number Lorentz transformation*

$T = t_2 - t_1$ can be calculated using the mixed number Lorentz transformation

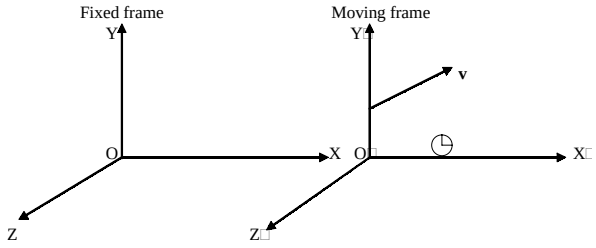

Figure 6

$$T = t_2 - t_1 = \gamma (t_2' + \mathbf{V.X}/c^2) - \gamma(t_1' + \mathbf{V.X}/c^2)$$

or, $T = \gamma (t_2' - t_1') = \gamma T_0$

or, $T = \gamma T_0$

This is the formula of time dilation of mixed number Lorentz transformation which is the same as that of special Lorentz transformation.

Relativistic addition of velocities

(i) *Relativistic addition of velocities of special Lorentz transformation.*

If we have a train moving with a velocity **v** with respect to ground and a passenger on the train moves

Table 1. Comparison of special, most general and mixed number Lorentz transformation.

	Special Lorentz transformation	Most general Lorentz transformation	Mixed number Lorentz transformation
(i)Length contraction	$L_0 = L\square$	$\mathbf{L}_0 = \mathbf{x}_2 \square \mathbf{x}_1 + \mathbf{v}[\{(x_2\cos\square \square x_1 \cos \square)\}/v\}](\square - 1)$	$L_0 = \square(\mathbf{L} - i\mathbf{L}\square\mathbf{v})$
(ii) Time dilation	$T = T_0\square$	$T = T_0\square$	$T = T_0\square$
(iii) relativistic addition of velocities	$\mathbf{u} = \dfrac{\mathbf{u}\square + \mathbf{v}}{1 + \mathbf{u}\square\mathbf{v}/c^2}$	$\mathbf{u} = \mathbf{v} + (1 \square v^2/c^2)^{1/2} \left[\mathbf{u}\square + \mathbf{v} \dfrac{(\mathbf{v}.\mathbf{u}\square)}{v^2} \{ (1 \square v^2/c^2)^{1/2} \square 1 \}\right]$	$\mathbf{u} = \dfrac{\mathbf{u}\square + \mathbf{v} + i\,\mathbf{u}\square\square\,\mathbf{v}}{1 + \mathbf{u}\square.\mathbf{v}}$

with a velocity $\mathbf{u}'$ with respect to the train, then the passenger's velocity $\mathbf{u}$ with respect to the ground can be calculated by the special Lorentz transformation, which can be written as

$$\mathbf{u} = \frac{\mathbf{u}' + \mathbf{v}}{1 + \mathbf{u}'\mathbf{v}/c^2} \qquad (24)$$

This is the relativistic or Einstein velocity addition theorem.

(ii) *Relativistic addition of velocities of most general Lorentz transformation*

Using the most general Lorentz transformation, the relativistic or Einstein velocity addition theorem can be written as

$$\mathbf{u} = \mathbf{v} + (1 \square v^2/c^2)^{1/2} \left[\mathbf{u}\square + \mathbf{v} \frac{(\mathbf{v}.\mathbf{u}\square)}{v^2} \{ (1 \square v^2/c^2)^{1/2} \square 1 \}\right] \qquad (25)$$

(iii) *Relativistic addition of velocities of mixed number Lorentz transformation*

Using the mixed number Lorentz transformation, the relativistic or Einstein velocity addition theorem can be written as

$$\mathbf{u} = \frac{\mathbf{u}' + \mathbf{v} + i\,\mathbf{u}' \times \mathbf{v}}{1 + \mathbf{u}'.\mathbf{v}} \qquad (26)$$

Conclusion

Length contraction, time dilation and relativistic addition of velocities of special Lorentz transformation, most general Lorentz transformation and mixed number Lorentz transformation are clearly explained. The explanation presented here will be helpful for the further application of mixed number Lorentz transformation.

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ON p-VALENT FUNCTIONS INVOLVING RUSCHEWEYH DERIVATIVES

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Abstract. The objective of this paper is to introduce some useful sufficient conditions for p-valent functions involving Ruscheweyh derivatives.

Keywords: Ruscheweyh derivative, p-valent, AMS (1991) Subject Classification.

Introduction

Let A(p) denote the class of functions of the form

$$f(z) = z^p + \sum_{k=p+1}^{\infty} a_k z^k \quad (p \in N = \{1,2,...\}) \quad (1.1)$$

which are analytic and p-valent in the unit disc U= {z : | z | < 1}.

For functions $f_j(z)$ (j = 1, 2) defined by

$$f_j(z) = z^p + \sum_{k=p+1}^{\infty} a_{k,j} z^k , \quad (1.2)$$

we define the convolution $f_1 * f_2(z)$ of functions $f_1(z)$ and $f_2(z)$ by

$$f_1 * f_2(z) = z^p + \sum_{k=p+1}^{\infty} a_{k,1} a_{k,2} z^k . \quad (1.3)$$

With the convolution above, we define

$$D^{n+p-1} f(z) = \left(\frac{z^p}{(1-z)^{n+p}} \right) * f(z) \quad (f(z) \in A(p)), \quad (1.4)$$

where n is any integer greater than –p. We note that

$$D^{n+p-1} f(z) = \frac{z^p (z^{n-1} f(z))^{(n+p-1)}}{n+p-1!} . \quad (1.5)$$

The symbol D^{n+p-1} when p = 1 was introduced by Ruscheweyh [5], and the symbol D^{n+p-1} was introduced by Goel and Sohi [1]. Therefore, we call the symbol $D^{n+p-1} f(z)$ the (n + p – 1)-th order Ruscheweyh derivative of f(z). It follows from (1.5) that

$$z\left(D^{n+p-1} f(z)\right)'' = (n+p)\left(D^{n+p} f(z)\right)' - (n+1)\left(D^{n+p-1} f(z)\right)' . \quad (1.6)$$

A function f(z) belonging to A(p) is said to be in the class $S_n(p)$ if it satisfies the condition

$$\mathrm{Re}\left\{ \frac{D^{n+p} f(z)}{D^{n+p-1} f(z)} \right\} > 0 \quad (z \in U) . \quad (1.7)$$

Further, a function f(z) is said to be in the class $C_n(p)$ if there exists a function g(z) belonging to $S_n(p)$ such that

$$\mathrm{Re}\left\{ \frac{\left(D^{n+p} f(z)\right)'}{\left(D^{n+p-1} g(z)\right)'} \right\} > 0 \quad (z \in U) . \quad (1.8)$$

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Since $g(z) = z^p$ belongs to the class $S_n(p)$, we observe that the function $f(z) \in A(p)$ satisfying

$$\mathrm{Re}\left\{\frac{\left(D^{n+p-1} f(z)\right)'}{p\, z^{p-1}}\right\} > 0 \qquad (z \in U) \qquad (1.9)$$

is a member of the class $C_n(p)$.

Recently, when n = 0, Nunokawa [3], Nunokawa and Owa [4], and Saitoh *et al.* [6] have proved some of p-valently close to convex, starlike and convex functions in the unit disc U.

Some Sufficient Conditions

In order to state and prove our results, we need to recall here the following lemma given by Jack [2].

Lemma 1

Let w(z) be regular in the unit disc U such that w(0) = 0. If |w(z)| attains its maximum value on the circle |z| = r at a point z_0, then

$$z_0 w'(z_0) = k\ w(z_0)\ ,$$

where $k \geq 1$ is a real number.

Applying the above lemma, we prove the following theorem.

Theorem 1

If a function f(z) is in the class A(p) and satisfies the condition

$$\left|\frac{\left(D^{n+p-1} f(z)\right)'}{p\, z^{p-1}} - 1\right|^{\alpha} \left|\frac{\left(D^{n+p-1} f(z)\right)''}{p\, z^{p-2}}\right|^{\beta} < 1 \qquad (z \in U) \quad (2.1)$$

for some $\alpha \geq 0, \beta \geq 0$, then $f(z) \in C_n(p)$.

Proof

Note that

$$\left|\frac{\left(D^{n+p-1} f(z)\right)'}{p\, z^{p-1}} - 1\right| < 1 \qquad (z \in U) \qquad (2.2)$$

implies (1.9). Therefore, we only need to prove that the condition (2.1) implies (2.2). Defining the function w(z) by

$$w(z) = \frac{\left(D^{n+p-1} f(z)\right)'}{p\, z^{p-1}} - 1 \qquad (z \in U) \qquad (2.3)$$

for f(z) belonging to A(p), we have that w(z) is regular in the unit disc U and w(0) = 0. It is easy to see that (2.3) leads to

$$\frac{\left(D^{n+p-1} f(z)\right)''}{p\, z^{p-2}} = (p-1)w(z) + zw'(z) + p - 1. \quad (2.4)$$

Therefore, the condition (2.1) becomes

$$|\, w(z)\,|^{\alpha}\, |\, (p-1)(1+w(z)) + zw'(z)\,|^{\beta} < 1. \quad (2.5)$$

Suppose that there exists a point $z_0 \in U$ such that

$$\max_{|z| \leq |z_0|} |\, w(z)\,| = |\, w(z_0)\,| = 1\ . \qquad (2.6)$$

Applying lemma 1 and letting $w(z_0) = e^{i\theta}$, we obtain

$$|\, w(z_0)\,|^{\alpha}\, |\, (p-1)(1+w(z_0)) + z_0 w'(z_0)\,|^{\beta}$$

$$= |\, (p-1) + (p+k-1)\, w(z_0)\,|^{\beta} \geq 1 \quad (k \geq 1) \quad (2.7)$$

which contradicts (2.5). This implies that |w(z)| < 1 for all $z \in U$, that is, $f(z) \in C_n(p)$.

Taking $\alpha = 0$ in Theorem 1, we obtain the following corollary.

Corollary 1

If a function f(z) is in the class A(p) and satisfies the condition

$$\left|\frac{\left(D^{n+p-1}f(z)\right)''}{p\,z^{p-z}}\right|^{\beta} < 1 \qquad (z \in U) \tag{2.8}$$

for some $\beta \geq 0$, then f(z) $\in$ $C_n(p)$.

Next, the following Theorem is in order.

Theorem 2

If a function f(z), is in the class A(p) and satisfies the condition

$$\left|\frac{\left(D^{n+p-1}f(z)\right)'}{p\,z^{p-1}} - 1\right|^{\alpha} \left|(p-1) + \frac{\left(D^{n+p}f(z)\right)'}{\left(D^{n+p-1}f(z)\right)'}\right|^{\beta} < \left(p + \frac{1}{2(n+p)}\right)^{\beta} \tag{2.9}$$

for some $\alpha \geq 0$, $\beta \geq 0$, and z $\in$ U, then f(z) $\in$ $C_n(p)$.

Proof

Consider the function w(z) defined by (2.3). Then, it follows that

$$p - 1 + \frac{\left(D^{n+p}f(z)\right)'}{\left(D^{n+p-1}f(z)\right)'} = p + \frac{zw'(z)}{(n+p)(1+w(z))} \cdot \tag{2.10}$$

Therefore

$$\left|\frac{\left(D^{n+p-1}f(z)\right)'}{p\,z^{p-1}} - 1\right|^{\alpha} \left|(p-1) + \frac{\left(D^{n+p}f(z)\right)'}{\left(D^{n+p-1}f(z)\right)'}\right|^{\beta}$$

$$= |w(z)|^{\alpha}\left|p + \frac{zw'(z)}{(n+p)(1+w(z))}\right|^{\beta} < \left(p + \frac{1}{2(n+p)}\right)^{\beta}. \tag{2.11}$$

Assuming that there exists a point $z_0 \in$ U such that

$$\max_{|z|\leq|z_0|} |\,w(z)\,| = |\,w(z_0)\,| = 1\,,$$

we can write $w(z_0) = e^{i\theta}$. With the aid of lemma 1, we observe that

$$|w(z_0)|^{\alpha}\left|\,p + \frac{zw'(z_0)}{(n+p)(1+w(z_0))}\,\right|^{\beta}$$

$$= \left|p + \frac{kw(z_0)}{(n+p)(1+w(z_0))}\right|^{\beta} \geq \left(p + \frac{1}{2(n+p)}\right)^{\beta}, \tag{2.12}$$

which contradicts (2.11). Thus, we conclude that f(z) $\in$ $C_n(p)$.

Letting $\alpha = 0$ and $\beta = 1$, Theorem 2 yields the following corollary.

Corollary 2

If a function f(z) is in the class A(p) and satisfies the condition

$$\left|p - 1 + \frac{\left(D^{n+p}f(z)\right)'}{\left(D^{n+p-1}f(z)\right)'}\right|^{\beta} < p + \frac{1}{2(n+p)} \qquad (z \in U), \tag{2.13}$$

then f(z) $\in$ $C_n(p)$.

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Review

NOVEL VISTAS OF GENE TRANSFER TO CEREALS

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Abstract: During the past decades, significant and far-reaching developments have been constantly taking place in the philosophy and methodology of gene transfer to cereals. These include cytogenetic manipulation of chromosomes such as gene transfer by direct recombination, molecular versus generative gene transfer, modification of chromosome affinity, indirect gene transfer via addition and substitution, gene transfer from alien addition or substitution chromosomes, homoeologous recombination, reciprocal translocations and centromeric translocations. Other significant approaches to gene transfer include: direct gene transfer into protoplast, biolistics, microinjection, agroinfection, liposome injection, liposome fusion with tissues and protoplasts, macroinjection and electroporation into tissues. Transgenic cereals (rice, maize) have so far been recovered exclusively by direct gene transfer applied to protoplasts. However, this method is not applicable to all varieties of all plant species. At present, *Agrobacterium*-mediated transformation is the choice method for introducing genes into the plant genome because of its genetically stable transformation, low copy number and easy manipulation *in vitro.* This method is also less prone to methylation and thus silencing.

***Keywords*:** Chromosome manipulation, DNA markers, biolistics, microinjection, *Agrobacterium*, integrative transformation.

Introduction

With an increasing number of plant genome projects now being completed, we have entered into an exciting era of crop genetic engineering [1]. Whole genome sequencing projects have been producing vast amount of potentially informative data for about eight years [2], yet there is a paucity of knowledge regarding the function of novel genes in cereals, particularly associated with economically important traits (often termed "quantitative trait loci – QTLs"). Recent functional analyses have emphasized analyses at the level of gene expression (transcriptomics), protein translation (proteomics) and the metabolic network (metabolomics) bridging the genotype-to-phenotype gap [3]. In the past most plant breeding programmes consisted of the selection of a desirable combination of alleles derived from related cultivars (the primary gene pool), and a slightly more distant form of the same species or occasionally even a different but closely related species (the secondary gene pool). In fact, simple selection after hybridization within the primary or with the closely related secondary gene pool is insufficient. The transfer of genes or complexes of genes from one species to another across effective barriers against genetic exchange (the tertiary gene pool) requires special measures whereby cytogenetic approaches can sometimes give a solution. Some of these inviolve "chromosome manipulations" [4,5] . An alternative for gene transfer across regular recombination barriers is *gene mutation*. This is not always successful when very specific, especially where dominant alleles are desired. In programmes to introduce genes from other forms, mutations have an application in removing epistatic gene, or the

original alleles when this is dominant and not removed by homologous exchange or even by homoeologous exchange.

Molecular Versus Generative Gene Transfer

Gene transfer from outside the primary or readily accessible part of the secondary gene pool is the field in which *in vitro* cell biological and molecular approaches offer very promising possibilities. The great interest in gene transfer by molecular techniques [6,7] has considerably reduced the interest in cytogenetic methods for transferring genes between species. Another reason for the decreased interest in cytogenetic techniques for gene transfer is that disappointing experience (especially the rapid breakdown of introduced disease resistance) has made plant breeders hesitant. One important difference with respect to the final chance of success between molecular/cell biological and cytogenetic techniques is the very large input available for the former and the very modest facilities for the latter approach. With the development of several new methods to monitor the transfer of recessive and hypostatic genes, the interest in the potentially very successful method of generative gene transfer may be expected to increase.

Identification of Transferred Gene

There has been considerable progress in the use of molecular markers that are closely linked to the gene to be transferred. These are much simpler to find and to clone than a gene that has not yet been identified on a molecular basis. Especially Restriction Fragments Length Polymorphism (RFLP) markers are useful to monitor the presence of genes in all forms of gene transfer. It seems reasonable to expect that in the future molecular, cell biological and generative cytogenetic techniques can be combined profitably to provide very effective facilities for gene transfer [5]. In several, but still a limited number of crops species, molecular markers are available on sufficient scale. Cereals of high economic importance such as maize [8], Wheat [9,10,11,12,13,14], Rice [15,16], barley [17] are particularly rich in the availability of such molecular markers.

When large numbers of RFLP markers are available in the critical chromosome segment, it is possible to establish the approximate size of the segment transferred. This may be important when; in addition to the target gene, less desirable genes are simultaneously transferred and are not directly separated from the target gene by backcrossing.

Instead of using molecular probes as markers of the genes themselves or as linked markers in genetic segregations, *in situ* hybridization, provides a probe of sufficient length. In such cases the transferred segment is at least several kb long. It may mark a gene transfer from one species or one genotype to another. The unplanned introgression of large chromosome segments of rye into wheat has been made visible by the use of a molecular marker specific for rye, which hybridized only to the rye segment [18]. Although single copy genes cannot yet be made visible by *in situ* hybridization in plants, low copy number repetitive genes have been located in somatic chromosomes. Multiple copies of originally single copy genes are often introduced by molecular transformation and can then be made visible [19]. They occur naturally in several instances; such as tDNA, seed storage protein genes [20].

Finally, monitoring of transferred segments, provided they are of sufficient size and sufficiently differentiated from the host, is possible by testing meiotic pairing with marked, known chromosomes. Using telocentrics of chromosome 7 of wheat, Eizenga [21] concluded that a

segment of chromosome 7 of *Agropyron elongatum*, carrying *Lr19* for resistance to *Puccinia recondita* rust, was on chromosome 7A and not on 7D as assumed earlier. The segment had been transferred by homoeologous recombination and was apparently sufficiently differentiated to show preferential pairing.

Gene Transfer by Direct Recombination

With sufficiently high levels of chromosome pairing affinity, artificial introgression of an alien gene from a related species into a chromosome of a cultivated species can often be realized simply by hybridization followed by straight recombination and recovery of most of the original genomes by cycles of backcrossing. Khush [22] reports success for rice with hybridization with exotic genetic source that lead to upsetting the meiotic pairing and exchange system in the hybrid (disruptive mating). This results in breaking up repulsion phase linkages in addition to the transfer of a gene to a hom(oe)ologous chromosome. These linkages would normally prevent the introduction of desired alien genes without linked undesirable genes. Change of the genetic exchange pattern in species hybrids with less drastic consequences has been reported by Jones [23] for two sub-species of rye, *S. cereal dighoricum* and *S. cereale turkestanicum*. Both have distal chiasma localization, but in the hybrid chiasma formation was close to random, except for normal, positive interference. It was possible to isolate progeny lines with the original parental chiasma distribution in addition to lines with random distribution. Apparently, there was segregation of genes for chiasma localization.

In interchange T240W (3R/5RL) of rye, the gene for brittle stem (*br*), located in the short arm of 5R, cannot be separated from the interchange by normal crossing over in the heterozygote. A telocentric 5RS combined with the interchange, however, appears to pair with the interchange chromosome 5RL/3R much more effectively than a complete chromosome 5R. As a consequence, recombination between the locus of *br* in the telocentric and the translocation break point is sufficiently frequent for the recovery of recombinants [24]. In cases where straightforward recombination between different species is possible it is necessary to restrict the size of the segment transferred. An example is the transfer of an interstitial segment of chromosome 1D, containing the gene *GluD1*, to rye chromosome 1R in *triticale*, replacing the corresponding rye gene *Sec-3*. *GluD1* is an important gene for bread making quality, normally not present in *triticale* [24].

When genes are introduced into chromosomes of an allopolyploid by homoeologous recombination, it is expected that most transfers will take place between the alien genome and the most closely related genome of the recipient. This, however, is not consistently so. Eizenga [21] reports that the transfer of the *Lr19* gene for *Puccinia recondita* resistance, transferred from *Agropyron elongatum* into wheat had in fact been to chromosome 7A in at least one case. In most cases, however, the transfer of genes from *A. elongatum* is to the D-genome of wheat, in accordance with the close relationship between the two genomes [25]. Goel and Saini [26] reported effectiveness of nine *Triticum tanschii* (Syn. *Aegilops squarrosa*) derived *Lr* genes in conferring resistance to nine prevalent leaf rust (*Puccinia recondita tritici*) races at seedling and adult stage of wheat.

Indirect Gene Transfer via Addition or Substitution

In order to circumvent the complications encountered with direct introgression from a distant species, indirect methods have been designed, primarily using the generative phase. The principal difference to the techniques discussed above is the introduction of an intermediate step consisting of first transferring a single entire chromosome from the donor to the recipient and subsequently transferring the target gene or a chromosome segment with this gene to the recipient.This eliminates the complication of disturbing the host genome by random

introgression of numerous genes from several chromosomes simultaneously with the desired gene. The largest number of gene transfers from an alien into a cultivated species by using addition [27,28] or substitutions [29] has been realized in wheat, in triticale [30], and maize [31]. In oats (*Avena sativa*) some instances of applicable gene transfer have been reported [32,33]. Jena and Khush [34] reported alien addition from *Oryza afficinalis* to *O. sativa*. In diploids likewise, additions are also possible such as rye [35,36,37,38]. In all these cases successful transfer of a gene from the alien chromosomes to the host has been reported [24].

Gene Transfer from Alien Addition or Substitution Chromosomes

There are three main routes leading to the transfer of a gene from the addition chromosome to the recipient genome namely, homoeologous recombination, radiation-induced translocation, and meiotic centromere translocation. The route chosen depends on the nature of the material. For instance, in monosomics, additions and substitutions the transfer of genes from alien addition or substitution chromosomes to recipient chromosomes has been applied primarily to allopolyploids and especially to wheat, where a large array of cytogenetic types and methods is available in diverse genetic backgrounds [39].

Homoeologous Recombination

A homoeologous substitution is much more favourable than an addition because of both the alien and the host chromosome only one copy is available, which avoids preferential pairing between two homologous host chromosomes. There are several examples. For instance of Knott [25] reported the homoeologous recombination between a chromosome of *Agropyron elongatum* (substituted for one chromosome 7D of wheat), and the remaining chromosome 7D, transferring stem rust resistance to wheat. The substitution was spontaneous, originating in the backcross progeny of the hybrid between the two species selected for rust resistance [40]. However, homologous pairing is not restricted to the specific chromosome pair involved, but involves all chromosomes of the host at the same times. The unintended induction of (homoeologous) pairing between the recipient genomes in the addition or substitution is hardly less disturbing than inducing homoeologous recombination in the hybrid. It is much less so in species where the initial pairing differentiation is more pronounced and not genetically enhanced [39].

Reciprocal Translocation

As early as 1956, Sears published a report on the transfer of dominant rust resistance from diploid *Aegilops umbellulata* to allohexaploid bread wheat via the addition of the *Aegilops* chromosome carrying the gene [27]. The approach developed by Sears to gene transfer has been applied in several more instances. One example is the transfer of rust resistance from *Agropyron intermedium* to wheat by Knott [41]. Wienheus [42] compared the practical suitability of addition, substitution and translocation of chromosome segments. Surprisingly the additions had a reasonable yield even equaling that of the control. The instability of the extra chromosome was the bottleneck. Translocations, as in the case of Sears [27], were the most promising, but quite laborious to isolate in sufficient numbers. There were several types, some apparently interstitial, and a few appeared practically useful. Alien gene transfer by translocation from an addition chromosome in allopolyploids has not only been successful in wheat. Mildew resistance, for example, has been transferred from *Avena barbata* to oat [32].

Centromere Translocation

The second method of using translocation of chromosome segments was referred by Sears [43]. It is based on a translocation at the centromere between the donor chromosome and a homoeologous recipient chromosome spontaneously formed after centromere break during the first anaphase of meiosis when both chromosomes are univalent. In principles, a centromere translocation can be reciprocal, but in most instances it is not. Centromere translocations have strictly defined break points. When the translocation is between homoeologous chromosomes, fully corresponding homoeologous segments are exchanged. Unlike with radiation-induced translocations, where the break points are not defined, single centromere translocation chromosomes between homoeologous chromosomes can be almost as functional as normal chromosomes because there are no duplications or deletions. Therefore, although most meiotic centromere translocations will be non-reciprocal, when they are formed between homoeologous chromosomes, there is a segmental homoeologous substitution and not a duplication deficiency.

In several wheat varieties into which mildew resistance has been introduced from triticale (triticale resistance), either an entire chromosome 1R from rye appeared to have been introduced as a substitution, usually for 1B or the short satellited arm 1RS had been translocated to the wheat 1B long arm with the break at the centromere. Zeller [44] concluded that there are two main origins for the several known cases of "Spontaneous" substituted and translocated 1R/1B chromosomes in wheat: the plant breeding institutions in Weihen – Stephan near Munich, and Salzmude near Halle, both in Germany. Also in several varieties listed by Zeller [44] that had one of the mildew resistance varieties from these institutions in their ancestry and that carried substitution or centromere translocations. Another example of centromere translocation between a rye and a wheat chromosome, is that of the stable transfer of Hessian fly (*Mayetiola destructor*) resistance from "Chaupon" rye to wheat via a 2BS/2RL translocation [45].

Centromere translocation between specific chromosomes can be formed in a monosomic substitution, where both the alien and the host chromosomes are univalent in meiotic anaphase. King *et al.* [46] made the monosomic substitution of 4SI from *Aegilops sharonensis* (the "Cuckoo" chromosome) for 4D (carrying the semi-dominant dwarfing gene Rht2). Four families derived from 594 BC1/F2 plants, had 42 chromosomes and were semi-dwarf with the "Cuckoo" segment combined with the Rht2 gene in one 4SI/4D translocation chromosome. Two of these four families had formed after centromere translocation, but the other two after non-centromere translocation.

Ren *et al*. [47,48] crossed octaploid triticale (AABBDDRR) with the parental wheat (AABBDD) and selfed the hybrid (AABBDDRR). In addition to rye chromosomes, which were necessarily univalent, some wheat chromosomes were lost also, apparently because they were univalent. Several translocations occurred among 837 progeny plants, 64 wheat/rye translocations and 256 rye/rye translocations were found. When specific wheat and rye chromosomes are made univalent to produce centromere translocations it is important to be sure that the arms involved are sufficiently homoeologous. Naranjo and Fernandez Rueda [49] showed that in the evolution of rye and wheat several translocations had altered the original composition of the chromosomes.

There is a clear variation between species with respect to centromere breakage and the formation of translocations, and, within wheat, also between genotypes [50] and between individual chromosomes within varieties [51]. All three methods discussed for transferring chromosome segments from alien chromosomes to their homoeologues in the recipient species even when properly applied, have their merits and demerits [39].

The additions of wheat chromosomes to diploid rye (*Scale cereale* 2n=14+1; [37, 38] are alloplasmic

because they have originated from a hybrid between triticale, which has wheat cytoplasm, and rye as the pollen parent. The genome composition of this hybrid is ABRR or ABDR if the triticale parent is octoploid triticale. In the backcross with rye the additions were recovered. These were identified by using C- and N- banding and by isozyme studies [37,38].

Schlegel and Kynast [38] report the successful transfer of a segment of chromosome 6B of wheat to rye by premeiotic irradiation with 1000 rad X-rays and using the pollen of the addition line to fertile normal rye. This simultaneously transfers the genome to rye cytoplasm and selects the most viable translocations. The F_1 was screened for alien chromatin by N-banding and chromosome pairing. Claus and Pohler [52] and Pohler *et al.* [53] describe the mitotic elimination of rye chromosomes from barley/rye hybrids as a result of anaphase lagging and subsequent disintegration. As a consequence some of the DNA or chromatin was incorporated into the barley genome and expressed. This resembles the transfer of genes and chromosome segments from the irradiated into the untreated nucleus after asymmetric fusion [54].

Gene Transfer Through Genetic Engineering

Genetic engineering is a direct manipulation of genetic material and it involves transfer of desirable gene(s) from one organism to another as well as transfers of new genes obtained by chemical synthesis. The discovery of restriction enzymes has allowed for the partitioning of the DNA molecule at certain points, each fragment being mostly an individual gene, and the recombining of these fragments into a new chain of recombinant DNA. A considerable number of genes extracted from plants have been isolated and cloned [55]. They are maintained in so-called gene libraries. One of the pioneer attempts was the transfer of nitrification genes (nif-genes) from *Rhizobium* bacteria to plants, which do not live in symbiosis with these bacteria, such as maize, wheat and other monocots. Besides viruses used as vectors, there are other methods of transferring a desirable gene into the recipient. Plasmids have been found suitable for that purpose, especially the Ti-plasmid (tumour inducing plasmid) which is isolated from a soil bacterium *Agrobacterium tumefaciens*. The plasmid may be cut by means of restriction enzymes and the desirable gene inserted into it. After that, the plasmid is linked by means of other enzymes, which has to be incorporated back into the bacterium, which is then used to infect the recipient organism. If plasmid DNA is integrated into the genome of the recipient and transferred genes shows its expression, it may be considered that a transgenic organism has been obtained.

Gene transfer by direct incorporation of Ti-plasmid into the protoplast is another possibility, which has been more effective in monocot plants [56]. The first results of direct transfer of genes for resistance to Kanamycin were obtained with protoplasts of *Triticum monococum* [57]. Microinjection of DNA into the nuclei of protoplasts or into inflorescences is also a new method of gene transfer, which has been demonstrated with Kanamycin resistance genes incorporated into floral tillers of rye [58]. The transfer of genes for resistance to antibiotics into plants is used only as a marker for other transfers, which might be useful in plant breeding. For example, the transfer of genes for resistance to herbicides would be an important achievement in agriculture. Direct injection into plants i.e., into seeds, embryos or pollen has been tried earlier [59]. There is recent evidence for transformation by direct injection of plasmid DNA into young floral tillers of rye [60], which succeeded in obtaining three transgenic rye plants [61].

Direct Gene Transfer into Protoplasts

Plants can be regenerated from isolated, single plant cells without cell walls – the protoplasts [62]. Protoplasts can be isolated from a great variety of plant tissues. As protoplasts are surrounded by only a single plasma membrane, it was expected that gene could be introduced directly with straightforward physical treatments. This was indeed the case [63], and within a few years the basic method was optimized such that transgenic plants could be produced with great efficiency either with electroporation [64] or with simple chemical treatment [65]. The plants showed perfect Mendelian inheritance of the foreign genes [66] and stabilities comparable to those of original plant genes. This method, called direct gene transfer, also enabled efficient co-transformation [67] and gene targeting [68]. As early as in 1989 plants were regenerated in about 150 species [69]. Also in many other species, cell cultures have been recovered from protoplasts. However, plant regeneration from protoplasts is very delicate and often unreliable process, depending upon too many parameters that are not under experimental control.

Microinjection

Microinjection uses microscopic devices and microinjection capillaries to deliver defined volume of DNA into defined cells without impairing their viability. This has been used with cereals to produce zygotic pro-embryos. The key problems would be the production of enough microspore-derived pro-embryo from the main cereals, and to the isolation of and plant regeneration from very young zygotic pro-embryos. Hundreds of microspore-derived pro-embryos of maize, wheat, rice and barley and of zygotic pro-embryos from these cereals have been microinjected and regenerated; many thousands of sexual offsprings have been analyzed for the presence of the foreign gene. Surprisingly, we have seen no case where we can prove that the foreign gene would have been inherited by the offspring. One problem is certainly the chimeric nature of putative transgenic plants, which makes transmission to offspring a chance event. We must also consider the possibility that cereal pro-embryos and meristems may not contain many cells competent for integrative transformation. The key problem for the development of an efficient gene transfer method for cereals may concern not the method of DNA delivery, but the availability of competent cells. This is where protoplasts probably have one of their great advantages over all other systems: protoplast isolation shifts potentially competent cells into the competent state. Information on microinjection of marker gene into microspore-derived pro-embryos produced transgenic chimeras [70] was readily available in 1987.

Particle Gun or Biolistics

The biolistics method has been developed from the rather surprising idea of bypassing all biological complications by shooting genes glued to heavy particles into plant cells [71,72]. The explosive force of gun powder or heated water is used to accelerate larger numbers of small metal particles into target tissues. This method has yielded evidence for transient expression of marker genes. The first case of regeneration of transgenic plants as a consequence of biolistic treatment is transgenic maize [62]. Since then, numerous laboratories, using biolistic devices in large-scale attempts, have found that this technology is not as efficient as expected. In fact, it is rather inefficient as far as integrative transformation is concerned. Why, however, is the biolistic approach so inefficient? We see several causes, and it will be difficult to alter them experimentally.

(i) The frequency of integrative transformation depends upon the concentration of the transforming gene, if this drops below a critical level, the transformation frequency is close to zero. It may be difficult to deliver enough DNA on a particle, or the DNA may be released too slowly from the particle.
(ii) Recovery of a transgenic plant requires that the foreign gene is transported into and

integrates in a cell that is competent for integrative transformation and for clonal propogation and regeneration. Such cells are probably extremely rare in cereals.

(iii) Even if successful, integrative transformation in one cell of a multi-cellular structure will yield a transgenic chimera, and if no secondary morphogenesis from and selection for those transgenic cells is easily possible, whether or not a transgenic sector will contribute to the "gene line" will depend on fortune.

We are therefore skeptical that biolistics can be developed into an efficient routine technique for every plant species.

Other Approaches

Agroinfection

The DNA transfer mechanism of *Agrobacterium tumefaciens* can be used for the transfer of virus genomes into plants where mechanical virus infection is not possible [73]. The virus then spreads systematically throughout the plants. This amplification of a single DNA transfer event has been used to study whether or not *Agrobacterium* can deliver DNA into cereals. Systemic spread of maize DNA virus in maize following agroinfection showed that this is indeed the case [74]. It was later shown that there is not much difference in the efficiency of DNA transfer between dicots and monocots [62].

Was this, then, the long expected proof that *Agrobacterium* is also a viable vector system for genetic engineering of cereals? Probably not. Using an engineered virus carrying a foreign gene, one could spread this gene in the individual agroinfected plant. As the virus is excluded from the meristems, it is excluded from transmission to the offspring. As the virus does not integrate into the host genome, there is little chance that it integrates into the genome of a cell of the "germ line" or one of the rare cells competent for transmission and regeneration. The only chance for integration of the foreign gene would be in wound-adjacent cells, which are neither competent nor viable.

Incubation of dry seeds

The most recent experiments have used dry cereal embryos separated from the endosperm at the scutellum, thereby creating a large wound site. Incubation in viral or non-viral DNA solutions yielded evidence of transient expression of marker genes and recombination of viral DNA [75]. Regeneration of plants from those embryos could lead to transgenic cereals [62]; a large number of offsprings were studied. There is so far no proof of integrative transformation, and the chances for transgenic cereals are extremely small; use of viral DNA may lead to systemic spread, which will not lead to integration; nonviral DNA has virtually no opportunity to reach competent cells that will contribute to shoot regeneration. The best this DNA can do is to reach some of the wound-adjacent cells, but these will not proliferate, and they die.

Liposome fusion with tissues and protoplasts

Fusion of DNA-containing liposomes with protoplasts is an established method for producing transgenic plants [76]. It has, however, no obvious advantage over direct gene transfer. DNA containing liposomes have also been applied to various tissues, cell cultures and pollen tubes, with the rationale that liposomes might helps transport the DNA via plasmodesmata or directly across the cell wall. Liposomes can carry small dye molecules into cells within tissues via fusion with the plasmalemma [77]. There is, however, no proof of transport and integration of marker genes. As plasmodesmata are sealed off immediately on wounding, this route is not open, even for small liposomes; impregnation of the cell wall with phospholipids seems not to change its barrier function.

Macroinjection

Use of injection needles with diameters greater than cell diameters leads to destruction of the cells. DNA integration would require that the DNA move into wound-adjacent cells. The most promising data so far were reported in an experiment where a marker gene was injected into the stem below the floral meristem of rye (*Secale cereale*) [60]. Hybridization to the marker gene and enzyme assays with selected sexual offspring yielded strong indicative evidence. Unfortunately, it has not so far been possible either to reproduce these data in several large-scale experiments with other cereals or to establish proof with the original material. It is also very difficult to understand how the DNA could reach the sporogenic cells in this experimental design, as DNA would have not only to reach neighboring cells but also to travel across many layers of cells.

Pollen maturation

Three key problems may have prevented pollen transformation so far: the cell wall, nucleases, and the intense heterochromatic state of sperm cells. The latter two problems may be overcome by the approach of *in vitro* maturation [78]. Immature microspores can be matured to functional pollen *in vitro*. If genes could be transferred at the microspore stage, they might have a chance for integration.

Electroporation into tissues

Discharge of a capacitor across a cell containing protoplasts and DNA solution is a routine method for DNA uptake as well as for stable integrative transformation [64]. Electroporation has also been applied to a variety of walled plant cells and tissues. The results so far confirm that cell walls are very efficient barriers against DNA molecules of the size of a functional gene [79].

Agrobacterium-mediated transformation

Agrobacterium-mediated transformation is the choice method for introducing genes into the plant genome because of its genetically stable transformation, low copy number, easy manipulation in vitro and appears to be less prone to methylation and thus silencing [80]. *Agrobacterium*-mediated gene transfer were established unequivocally in rice (*Oryza sativa* L., [81]), barley (*Hordeum vulgare* L., [82]), maize (*Zea mays* L., [83]), Sorghum (*Sorghum halepense* L., [84]) and wheat (*Triticum aestivum* L., [85,86]).

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Review

QUALITY IN HIGHER EDUCATION

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Introduction

In order to improve standards of living, the performance of organizations in public and private sector will depend increasingly on quality of education provided to the citizens in the form of values, knowledge, and learning skills. These elements determine how effectively people will function in a complex and uncertain environment and the satisfaction they will drive from their work and other activities [1]. According to the Japanese, 'quality begins and ends with education' and also the quality improvement in education system is the key to quality future [2].

In industry it has been increasingly recognized that a high quality of product and its associated customer satisfaction is one of the key factors for the survival of any enterprise. At university we have so far been looking at excellence in education as directly correlated with matters such as degrees, professional experiences, and authorship and research activities. It is usually argued that quality could not be measured but the academicians can recognize it when and where it exists.

By and large the concept of quality management has been considered alien by the university communities. Quality of higher education is no longer a national concern but has become an international issue through academic, political, and commercial development associated with globalization [3]. Increasing pressures from funding authorities and external criticism by stakeholders are some of the factors which are likely to make university community less reluctant to change. A careful review of Total Quality Management (TQM) as applied to education may assist to making it acceptable to the universities. Adopting TQM is considered to be the first step needed for implementing quality improvement in institutions of higher education.

Requirements for TQM in Education

TQM is a management philosophy based on the principle of satisfying customers, obsession of quality, involvement of everyone in the organization, and continuous improvement [3,4,5]. The needs of students and other stakeholders are required to be gauged. Figure1 depicts the stakeholders of higher education. Feedback from students regarding their educational experiences and concerns of other stakeholders are major indicators to bring about improvement wherever possible. Higher Education Commission, Government of Pakistan, is trying to provide increased resources to public institutions so that the students have the latest technologies and methodologies available. Students needs in terms of support staff/services and extracurricular activities also need to be taken into account. The university management must examine the ways resources are used to bring about the changes necessary for continuous improvement of education process. This

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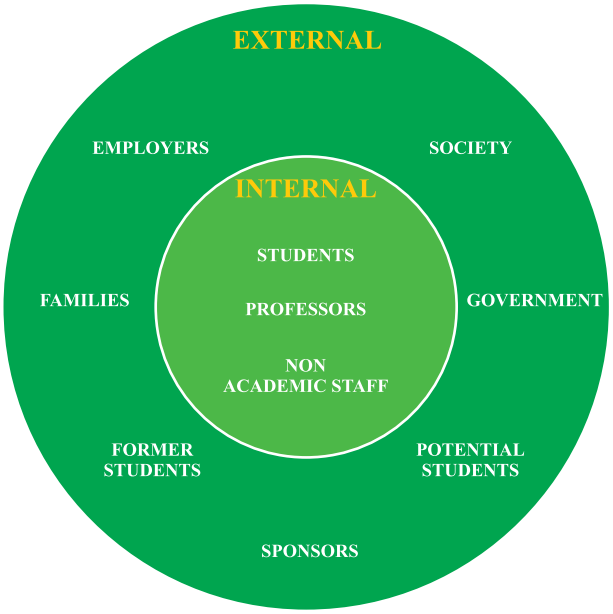

Figure 1. Stakeholders of higher education.

means providing finances for faculty and staff development and providing the equipment and support services needed to improve the learning process on a continuous basis. These things involve increased funding for higher education, and administrators must lead the way in convincing the authorities concerned that the resources are essential on continuous basis before benefits of change can be realized.

TQM in education

The needs of students and other stakeholders must be established to maintain the central purpose of higher education. Figure 2 shows the four factors that affect the quality of education [6].

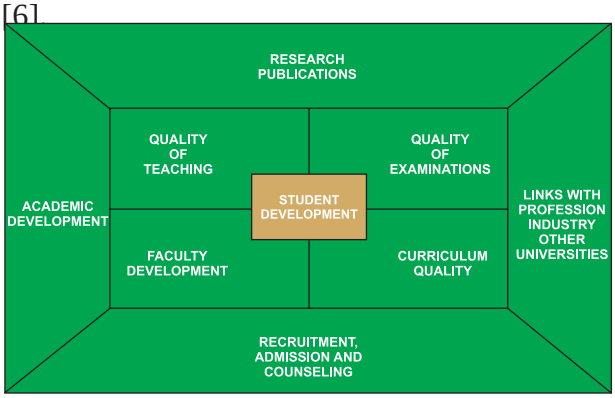

Figure 2. Factors effecting quality in higher education.

A main consideration must be that the teaching/learning process is central to education. The whole process should be geared toward meeting the needs of students while looking to the future and envisioning how those needs will change. Care must be taken When reviewing those needs and asking students for recommendations,. For example, narrow uses of customer service applications may be inappropriate. It would be improper to use suggestions from students concerning the content of curriculum if their background and experiences are limited. Quality of instruction can be looked at by reviewing the processes and by obtaining feedback from students.

Other needs from stakeholders, including peers and those who are external to the institution, must also be considered. This aspect remains important in determining content, diagnosing students and assessing educational outcomes. The Faculty must analyze and determine how technological advances will be used as new methods of instruction and advanced ways to help students achieve their objectives. It is vital that TQM focus on continuous improvement in our universities is maintained.

Total quality management puts systems and processes in place to meet and exceed the expectations of customers. It is a relentless quest for continuous improvement through documentation and the use of tools in a problem-solving atmosphere that features team action and good leadership practices.

Improvement of systems through process management, then, is a key feature of TQM. The goal is to improve systems and processes by creating an environment where faculty and staff examine customer needs and perform their activities in the most efficient manner possible. Faculty development is vital to ensure that

teaching staff remain apprised of new theories and teaching methodologies so that they are creating new values for the future, instead of communicating the values of past. Administrators and staff need to be educated in how to be more increasingly efficient in their respective positions. Administrators, faculty and staff need training in TQM concepts, tools and techniques to assist them in knowing their roles and responsibilities in this quest for continuous improvement.

The best place to start is to improve communication lines and ensure that everyone is more comfortable with ones work situation. The Faculty and staff must be encouraged to inform administrators about situations in which process improvement can occur. Administrators must remove barriers which stand in the way. It must be recognized that the institution will benefit when people are empowered to improve themselves. This involves a professional development focus and total support for all. This administrative support includes a firm commitment to implement and support a total quality improvement initiative. This involvement and commitment are essential because TQM is a long range, rather than a short term process. Faculty and staff need assurance that total quality is not just another administrative program which will wither and die. TQM theories and concepts will fit in higher education when all become convinced that their application can be customized to the institution itself and that everyone must become involved in its implementation and success. In developed countries universities of good standing are adopting TQM and number of such universities is continuously increasing.

Concepts of Quality in Education

When work is viewed as a process, it is easy to understand that improvements will come when inputs are received from everyone involved in each segment of work. In this new people-centered atmosphere, those who deliver the work and perform the processes define quality according to standards which are set and are based on those who use or benefit from the product or service being delivered. Yorke [7] has defined the standards for education as shown in Figure-3. It is the customer expectations or requirements that need to be met. This customer first approach is rather alien to education for the focus in the past has often been on self rather than on others. Professors normally feel that they know what is best for others. The TQM ethic, however, focuses on service to others.

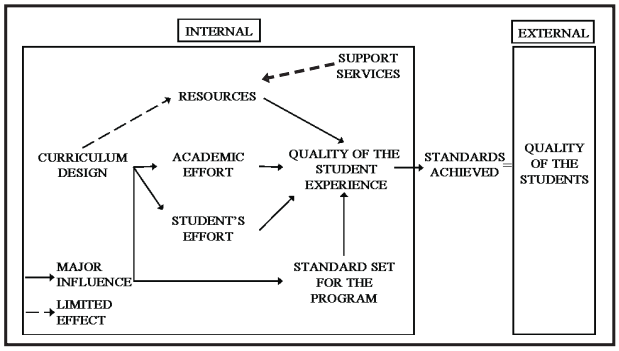

Figure 3. Quality and standards in higher education.

Customer Focus

Education is a service with customers like any other business, and those customers express satisfaction and dissatisfaction about services and instruction. When TQM is applied to education, it is essential that customers be identified by the supplier and that processes be established in order to determine their specific needs. The quality of services is then continually analyzed as efforts are made to meet and exceed customer expectations. Educators must learn what constitutes quality in the eyes of its past, current and potential customers and then deliver what is necessary to meet and exceed those expectations. To define quality, you have to ask those people who your products or services are intended to benefit. Customer service compels organizations to be specific about those they serve.

Who is education intended to benefit? Students are the primary customers, to be sure, but the customer relationship is somewhat different from a customer in a bank or a restaurant. Students may

not know what they need to learn. That is where the professional educator comes in. The teacher can observe the wants and needs of students, then balance those with the needs of other customers who may also have a stake in the education program and the future graduates. These include employers who hire graduates and other educators who may later provide advanced instruction. The professor in this process applies expertise in meeting and exceeding the expectations of the various stakeholders (customers) and determining how best to do it, given the desires and constraints imposed by them all. Likewise, the instructor is a customer or supplier for internal processes too. A teacher is a customer of other educators as he/she cooperates to integrate the curriculum of the specific discipline. The teacher receives services from others within the university. In this scenario, all the service department staff are suppliers as they provide services to both students and instructors. The ultimate goal is to improve processes and

Table 1. Customer supplier relationship in higher education system.

CUSTOMERS	SUPPLIER	SERVICE
STUDENTS	FACULTY MEMBERS	CURRICULUM DESIGN
		LEADERSHIP
	ADMINISTRATORS	MATERIAL & EQUIPMENT
		SYSTEM DEVELOPMENT
		MATERIAL & EQUIPMENT
	GOVERNING BODY	POLICY
PROFESSOR	ADMINISTRATORS	MATERIAL & EQUIPMENT
PARENTS	HIGHER EDUCATION SYSTEM	KNOWLEDGE, WISDOM, KNOW HOW, CHARACTER
INDUSTRY	HIGHER EDUCATION SYSTEM	KNOWLEDGE, WISDOM, KNOW HOW, CHARACTER
FACULTY MEMBER	FACULTY MEMBER	KNOWLEDGE, WISDOM, KNOW HOW, CHARACTER
FACULTY MEMBER	STUDENT	FEEDBACK ON LEARNING PROCESS

KNOWLEDGE WHICH ENABLES US TO UNDERSTAND WHAT WE LEARN IN RELATION TO WHAT WE ALREADY KNOW.

KNOW HOW WHICH ENABLES US TO DO, TO PUT OUR KNOWLEDGE TO WORK.

WISDOM WHICH IS THE ABILITY TO DISTINGUISH WHAT IS IMPORTANT FROM WHAT IS NOT. HELP TO ESTABLISH PRIORITIES.

CHARACTER WHICH IS A COMBINATION OF KNOWLEDGE KNOW HOW AND WISDOM ALONG WITH MOTIVATION

systems to exceed the needs of customers. The customers are, therefore, of two types: external (students, employers, the community at large, taxpayers, other educators from different institutions) and internal (other instructors, service department staff). Tribus [8] has developed a relationship between customers, suppliers, and services and these are shown in Table-1. Referring to students as customers does not necessarily mean they must be given whatever they request. A helpful analogy may be to view the administrators of the university as the supplier of services to faculty and staff. In that context, the faculty and the staff become customers of administrators.

Sometimes, even though administrators may wish to meet all the requests of the faculty and staff, not all needs can be met. Leadership comes in as administrators view requests for services and balance them with priorities due to prevailing constraints. A course of action is decided after weighing the requests and needs of all customers. It is the same leadership that the professor assumes when sorting out the requests and expectations of customer students and then balancing them with other stakeholders.

This customer focus and focus on continuous improvement also uses another idea of TQM—competitive benchmarking [9]. The best description of benchmarking is that it involves searching systematically for best practices and then adopting them. In higher education this idea should be attractive as there are several well-conceived initiatives and cost-effective approaches in place in educational institutions. The TQM process itself offers great opportunities for benchmarking and sharing successes and tribulations. While the goal is to have a TQM model unique to each institution, there are several ideas and activities which can and should be shared and replicated.

Throughout these processes of customer services, the leader administrator (or professor) can never relinquish responsibilities to sort out requests from various stakeholders, review those needs in light of existing circumstances, and then make decisions following input from many different sources. The professor assumes responsibility for determining appropriate course content and ensuring that student customers have needed opportunities to learn it. This compels the professor to define learning requirements and provide assurances that appropriate instructional/learning processes are in place and clear to all. This includes assurances that learning objectives are met through the application of tested teaching/learning strategies and methodologies.

This customer focus requires that a clear organizational mission and vision is in place to give purpose and a sense of accomplishment to it all. The main requirement is to have everyone focused on the central mission of the university. Each instructional program area and service department should also have its own written mission and purpose which complements the organization. This helps everyone to know what to aim for and how to prioritize and organize processes. Even in the classroom setting, a clearly stated mission and purpose by the professor will help students to learn how mission and institutional vision are used with input from various stakeholders who are a part of the complex network of the university.

Role of Leadership

Visionary leadership and demonstrated commitment must be provided for the TQM-process. Top-level support is essential to show everyone on the campus that the administration supports the quality improvement initiative. Besides this commitment to TQM, at the upper levels of the institution, assistance and support from the faculty in the various program areas are also required. This calls for the identification of priorities and design of action plans which are endorsed by the faculty and staff. Good communication and active involvement by all are the best way to make it work effectively.

The emphasis on leadership instead of traditional management changes the focus and transforms the culture of the TQM campus. Senior and middle managers and department chairs take on new roles which centre on shared decision-making with maximum input from faculty and staff. In this modified system, friendly collaboration and empowerment prevails in a less hierarchical and more integrated network.

This shift toward new leadership styles throughout the institution is a marked departure from traditional bureaucracies with top-to-bottom control. While those traditional approaches focused on accountability and authority, they usually lost effectiveness, efficiency and spirit because of the control imposed by those in charge. The TQM culture has faculty, staff and students owning their work and taking responsibility for learning with a shared mission and purpose. A non-threatening environment is sought through the sharing of power, ownership, authority and trust. The idea is to encourage leadership skills throughout the organization by mutual understanding of the mission of both the organization and the administrative units which creates recognition by all of what is needed to enhance this new culture. The leadership styles practiced by the senior executives become the leadership model for the classroom, as professors and staff exhibit the same skills. In this mutually supportive environment, professors and administrators become leaders as they provide instructions and services to students, faculty members and other staff. All parties begin to realize that their individual successes are interlocked with team action—their achievements rise and fall together. Traditional roles change as the need for new leadership styles becomes predominant in the TQM organization. In every department, classroom and work unit of the campus, envisioning, integration and enabling replace controlling, directing and executing.

Team Approach

The new TQM culture of the university requires renewed and genuine teamwork because significant and lasting changes will not occur unless professors and other staff are directly and actively involved in the planning and development of desired changes. Such involvement by people closest to the customers is paramount to the success of TQM in the institution. This requires everyone to be involved in quality improvement by participating as team members.

Teams are groups of people who work together toward common ends. They are the cornerstone of TQM. Teams can best review processes, determine the problems, find their root causes and eliminate them for ever. Teams have the expertise because members are usually closest to the customer. Teams solve problems by documenting the processes of the work they are involved in and building consensus around issues while eliminating the causes of the problems in a systematic way. In its simplest form, teamwork can be defined as joint action by a group in which each individual subordinates his/her interests and opinions to the unity and interests of the group. Teamwork is not *only desired, it is required if problems* are to be solved and meaningful changes are to occur in education.

A basic tenet of TQM, therefore, is that effective teams are stronger than the sum of its individual members. This requires a breakdown of subcultures usually prevailing in our educational institutions. These subcultures in the individual departments tend to develop policies and procedures which safeguard their own interests. Competition emerges as people are rewarded and departments are ranked based on comparisons with others. TQM seeks

improvement by reviewing and changing faculty systems and processes that cross department lines. An environment of cooperation is sought on the campus as teamwork from professors, executives, department chairs and other stakeholders identifies and analyzes problems and corrects them forever. In classrooms, laboratories and lecture halls the same problem-solving techniques are taught to students who are encouraged to work in teams and learn from each other.

The TQM systems approach seeks to improve the whole without ignoring individual department accomplishments. In an environment of continuous improvement, dynamic and proactive approaches with an eye toward interrelationships become the norm. This improvement model views the entire campus as a system with hundreds of processes which are subject to review and analysis. This philosophy of continuous improvement when applied to teaching and learning, examines all instructional processes. The entire system of curriculum and instruction, and all the other supportive processes commonly called student and/or instructional services, come under review by faculty and staff.

Process Management

The review of processes requires methods and tools common to TQM organizations. Teams address problems by applying the correct tools and scientific approaches in a shared decision-making atmosphere. The teams monitor the corrective solutions being tried, and standardize those which work in cooperation with others. In this environment of problem-solving, data and scientific methodology ensure that systems are designed carefully and faulty ones corrected in permanent ways rather than with temporary fixes. The goal is to eliminate the causes of the problem permanently.

There are several tools and techniques commonly used in problem-solving. These are known as the seven management tools and they include flow-charts, cause-and-effect diagrams, histograms, pareto charts, run charts, scatter diagrams and control charts [4]. Other effective tools and methods include surveys, focus groups and interviews with customers. Several planning tools are also used to create strategic directions and set goals in the institution and on teams.

A system of ongoing assessment is also required. This evaluation system examines the entire organization using agreed key performance indicators. These indicators become benchmarks for the university and targets for improvement as they are continuously readjusted in a dynamic environment of assessing customer needs and setting systems in place to meet and exceed expectations.

Data Requirement

Quality must be specific and monitored and that requires the use of meaningful and correct data in the TQM organization. In the TQM environment, the goal is to collect data which determines and documents customer needs. It deploys public, visible information systems to let each person and each team know how they are doing. The information is shared with faculty and staff in concise and meaningful reports which are used to improve systems and processes. After processes are reviewed and changed to meet customer needs, timetables are established to monitor processes and collect data on a departmental and campus-wide basis. This information system details the extent of improvement in the institution. The scope and validity of data and sharing of information are basic to TQM culture. A major shift occurs in data gathering, compilation and dissemination as everyone has access to information which in the past had been reserved for only a select few. This sharing is necessary as the information is used by people to help them make better decisions with meaningful data collected, used and analyzed by those closest to the processes and the customer.

Cultural Change

In the TQM institution, people are seen as its most important resource. Everything possible is done

to give faculty and staff the training, tools and initiative to contribute to its mission and goals. This creates cultural changes needed for meaningful reform in education. TQM becomes the model for systematic and continual improvement and change based on the needs of external and internal customers. New leadership skills emerge as the university's management and administrative styles come under review. A cultural revolution occurs across the campus as these changes occur and mutual accountability replaces individuality. This requires each institution to be a learning organization.

In such a mutually supportive environment, faculty and staff become even more directly involved in defining and monitoring the mission, purposes and strategic directions of the campus. This results in positive changes in morale and attitudes. Finger pointing usually stops and blaming ceases as a hostile and negative atmosphere is replaced with trust and mutual respect based on integrity and professionalism. There is an improvement in conditions for both students and staff. This human development feature of TQM will receive positive responses.

Unfortunately, human and organizational development consists of problem-solving activities which focus on accountability alone. The preferred system is to have a balanced approach which improves processes and accountability and enhances the welfare and morale of everyone associated with the changes being advocated. Such positive changes will set the stage for further improvement through team-building, consensus reaching and conflict resolution. All this will not happen quickly. It requires commitment and most of all patience. Administrators must loosen the management reins and give up control. That is difficult. Administrators have been rewarded in the past for the control they exercised, and as such changes at first will be gradual. There will be uneven application across the organization until these changes become permanent and meaningful, rather than temporary and cosmetic. People at all levels on the campus soon find out that a team effort requiring ongoing communications from all corners of the institution works best. They come to understand that there is a delicate balance between the application of the techniques, tools and strategies of TQM, and the human/social aspects which focus on respect, dignity and worth of all individuals. A leader who can strike a balance between these two applications will experience a successful cultural transformation with a gradual improvement in relationships. The overlying principle looks at everyone in the organization as being inherently competent. That philosophy changes everyone's outlook on the roles and functions of people in the university.

Professional Development

The primary factor which distinguishes TQM organizations from others is the focus on the individual development of employees. Those in charge realize that they can gain the competitive advantage over other organizations by having a learning workforce who are well trained and up to date. They know that a highly educated team of individuals who examine and change how work is processed and reviewed will set them apart from other institutions. Education and training programs should be based directly on the professional development needs of every individual from the vice chancellor of the university to the lowest personnel level on campus. Each person needs to be academically and technically competent in his/her specialty (teaching, managing, service, technical). The individuals have to be up-to-date in their specialty, for the university to become a complacent place.

The second component provides education in TQM concepts, language and technical skills.

This keeps the process going and links staff together toward a constant purpose. It reminds everyone about the institutional commitment to TQM and keeps individuals current in the topics related to the movement. The ideal program is one where everyone participates and there is emphasis on TQM application at each personnel level (teaching, administrative, service). This application feature is complemented with specialty topics related to TQM which are offered based on the individual's responsibilities and requirements.

The ultimate goal of the staff development program should be a plan which is comprehensive and individualized with integration of TQM instruction with other career enhancement aspects.

Quality Assessment

The commonly used mechanics or formats for quality assessment are;

- Malcolm Baldrige Standards
- European Foundation for Quality Management

The Malcolm Baldrige Standards [10] were developed to recognize companies in the US which excel in quality achievement and quality management. The award promotes awareness of TQM, an understanding of its requirements, and a sharing of successful strategies derived during implementation of TQM. The criteria used helps to summarize the strengths, determine areas for improvement and identify successful management styles. The criteria incorporate an objective means of evaluating an organization's total quality system. European Foundation for Quality Management EFQM model [11] is used in industry and universities and is a widely accepted tool for process improvement. This model includes enablers and results of an organization. This enables leaders to find out which enablers (leadership, policy, strategy, people management, resources, and processes) have to be taken into account and to be planned where improved results are desired.

It may be felt that standards or a set of measurable indicators of excellence are needed. However such standards may be the answer. The best criteria are the ones which are unique to the institution for which they were designed. While national policies which promote reform in education through TQM may work, it is felt that unique criteria created by the faculty, staff and customers of each institution will best serve the institution in its quest for excellence.

It is imperative that quality levels reached today won't be good enough tomorrow. It requires that indicators of excellence and institutional effectiveness must constantly be upgraded. Continuous improvement demands that the institution think systematically about the constant monitoring of processes valued by customers. The focus shifts to paying attention to the needs and expectations of others. This concept is so critical to quality that some experts choose to call the TQM process continuous process improvement or quality process improvement. This concept requires that universities be driven by motivation among the faculty and staff to do the best work possible, in an atmosphere of dynamic change.

Results

What results can a university expect from implementing TQM? What are the returns on the investment made to create this transformation in the institution? Are the results worth the time and money spent? These questions and similar ones will come up and need to be answered. There are no easy answers to such questions.

The problem is that results do not happen quickly. The up-front training which is needed is both expensive and time consuming and major changes will not happen and results will not improve until people are trained in the concepts, tools and techniques of quality.

A, great patience is needed. This is why top-level commitment is so vital. Otherwise, those early expectations of staff will create special challenges which might be hard to overcome. If there is enough patience, the results will begin to justify the early investments in training and staff time. It is felt that an organization will automatically improve and show results in time if the quality process is properly applied. In education, improvement occurs when administrators, faculty and support staff work together with other stakeholders to identify requirements, make necessary changes, measure the results and standardize the changes which show positive benefits. This is repeated throughout the institution as the staff uses continuous improvement strategies to eliminate non-conformance. Departmental results multiply as improvement happens in small increments throughout the University. In education, results can be examined in following areas as outlined in these questions:

1. Has learning improved as a result of TQM implementation?
2. Is the institution more efficient?
3. Has the culture of the institution changed with a primary focus on the needs of customers?

There should be criteria to examine the results of TQM implementation. The sum total of any institutional effectiveness plan must also reflect measurement in these areas. Those results should provide enough indicators to justify the quality initiative. An effort has been made to describe the TQM approach to educational institutions. It is hoped that in due course most of our universities will decide to adopt this approach.

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Short Communication

STUDIES ON FATTY ACID CONTENT OF SILAGE PREPARED FROM ORANGE WASTE AND MIXED VEGETABLE WASTE

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Abstract: Silage was prepared from orange waste, vegetable waste, and oat leaves separately, mixing with one another (1:1) and with milk curd after storing and fermenting for 90 days at ambient temperature (28-32°C) in sealed polythene bags. The biochemical changes of fatty acids and pH occurring in various samples were studied after 0, 30, 60 and 90 days. The result indicate that the change in capric, lauric, myristic and linolenic acids is very small But there is a remarkable change in palmitic, stearic, oleic and linoleic acids in various samples.

Keywords: Silage, fatly acids, orange waste, vegetable waste.

Introduction

Traditionally the silage, a highly nutritive animal feed, is being prepared from nontoxic herbages and fodder crops of high moisture content by fermentation under the controlled conditions. Under unsuitable conditions, foliage decays to useless inedible and toxic products poor nutritional value [1]. Improper storage conditions usually reduce the nutritive value of the products due to oxidation of fatty acids [2]. Some degradation of carotenes occurs during silage preparation by the action of enzyme (lipoxygenase) which oxidizes the fatty acids and lipids. The hydroperoxide radicals formed, oxidize the carotenoids and other oxylabile compounds [3]. Production of silage from various forages, action of plant enzymes, influence of oxygen, water, clostridia and other micro organisms has been discussed and reported by animal nutritionist [4]. Oxidative rancidity, thermal and oxidative polymerization have also been reported affecting the nutritive value of the products [5,6]. The conversion of banana waste, mango waste, vegetable wastes and maize fodder into a highly nutritive feed by anaerobic fermentation has also been described previously [7,8]. The present study on silage prepared from orange waste, vegetable waste and oat leaves thus is an extension of earlier studies.

Materials and Methods

Orange waste (OW) was procured from a juice making unit, vegetable wastes (VW) and oat leaves (OL) were taken from local market. The vegetables waste was a mixture of cauliflower, carrot, radish white and spinach. Orange waste, vegetable waste and oat leaves were chopped in a disc chopper (Hobert, England) separately. Six replicates of the eight samples (5kg each) having different composition OW, OW + VW (1:1), OW + OL (1:1), OW + 5ml milk curd (MC), OW +VW (1:1) + MC (5ml), OW + OL (1:1) + MC (5ml), VW and OL were packed in thick polythene bags, pressed, sealed and stored in a glass chamber at ambient temperature (28-32^{0}C) for further studies after an interval of 0, 30, 60 and 90 days.

Oil from the above samples (500g each) was extracted with n-hexane in a soxhlet apparatus [9] separately after 0, 30, 60 and 90 days. The hexane

Table 1. Fatty acid composition (%) of various silages when stored for different periods.

FattyAcids		Samples															
		1				2				3				4			
		Orange waste Days				Orange waste + Vegetable Waste (1:1) Days				Orange waste + Oat leaves (1:1) Days				Orange waste + 5 ml milk curd Days			
		0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90
Caprylic	$C_{8:0}$	9.4	–	–	9.3	–	–	–	9.2	–	–	–	10.5	–	–	–	
Capric	$C_{10:0}$	1.3	2.6	2.7	1.2	0.6	–	–	–	0.5	–	–	–	1.2	–	–	–
Lauric	$C_{12:0}$	1.0	2.7	1.5	0.4	1.1	1.0	0.2	1.7	0.6	1.2	1.0	1.6	0.8	0.9	0.5	0.6
Mystric	$C_{14:0}$	4.1	4.8	4.9	5.0	0.7	1.3	1.4	1.6	1.3	2.5	1.0	1.0	1.7	2.6	0.4	–
Palmitic	$C_{16:0}$	19.5	20.6	21.2	21.5	23.9	26.2	26.7	28.7	27.7	30.8	31.2	31.6	28.1	34.2	34.9	35.1
Palmitoleic	$C_{16:1}$	1.8	2.2	2.3	2.4	1.3	2.4	2.7	–	2.8	5.2	5.3	3.2	2.6	2.8	3.2	4.2
Stearic	$C_{18:0}$	2.8	3.1	3.5	3.7	3.5	3.7	3.8	4.3	3.0	3.2	3.5	3.1	2.4	2.6	2.9	1.3
Oleic	$C_{18:1}$	26.0	26.9	27.1	27.6	20.7	23.8	23.9	25.1	23.8	24.3	24.7	25.1	20.2	21.7	22.2	22.4
Linoleic	$C_{18:2}$	27.6	29.6	30.2	30.4	23.4	26.7	26.8	32.1	25.5	26.7	27.1	27.6	26.3	28.3	28.9	29.1
Linolenic	$C_{18:3}$	6.5	7.5	7.6	7.8	15.5	14.3	14.5	5.9	5.6	6.1	6.2	6.2	6.2	6.9	7.0	7.3

– Dry matter basis
– All values in the table represent average of six replicates

Table 2. Fatty acid composition (%) of various silages when stored for different periods.

FattyAcids		Samples															
		5				6				7				8			
		Orange waste + Vegetable Waste (1:1) + 5 ml milk curd Days				Orange waste + Oat leaves (1:1) + 5 ml milk curd Days				Vegetable waste Days				Fresh Oat leaves Days			
		0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90
Caprylic	$C_{8:0}$	8.4	–	–	–	8.7	–	–	–	10.0	–	–	–	10.0	–	–	–
Capric	$C_{10:0}$	0.2	–	0.3	–	0.6	–	1.2	–	1.7	1.8	–	–	1.6	0.6	–	–
Lauric	$C_{12:0}$	0.8	1.4	1.4	1.5	1.0	1.3	2.3	3.2	2.4	2.6	1.0	1.8	2.2	1.8	1.5	1.0
Mystric	$C_{14:0}$	0.8	1.6	3.8	4.2	3.7	4.6	8.7	9.6	2.0	2.8	2.9	3.5	2.0	2.5	2.7	0.1
Palmitic	$C_{16:0}$	22.1	23.9	34.8	40.1	27.0	31.4	36.8	37.5	31.2	31.3	32.6	33.8	30.4	32.5	32.8	33.3
Palmitoleic	$C_{16:1}$	1.5	2.5	2.9	3.3	4.3	5.2	6.9	7.1	3.8	5.7	6.0	–	3.6	5.2	3.1	2.0
Stearic	$C_{18:0}$	4.1	4.4	4.8	5.6	6.6	6.7	7.1	8.2	6.2	6.5	6.9	7.5	7.0	8.3	8.5	9.6
Oleic	$C_{18:1}$	29.2	30.1	30.9	31.2	25.4	29.5	31.4	32.6	16.4	18.8	23.8	34.0	15.7	18.6	19.0	21.5
Linoleic	$C_{18:2}$	27.3	26.5	21.1	14.1	16.2	17.8	5.6	1.8	20.7	23.9	19.6	11.4	21.1	23.8	25.4	25.4
Linolenic	$C_{18:3}$	5.6	7.6	–	–	6.5	3.5	–	–	5.6	6.6	7.2	8.0	6.4	6.7	7.0	7.1

– Dry matter basis
– All values in the table represent average of six replicates

extracts were dried on water bath at 70^0C to a constant weight. The oil obtained was preserved separately in desiccators for further studies. Oil samples were treated with boron-trifluoride methanol reagent for half an hour in test tubes with teflon lined screw caps on water bath separately. The methyl esters so formed were extracted with n-hexane and the solvent was removed by distillation to get pure methyl esters for gas liquid chromatography [10]. Methyl esters of the fatty acids were analyzed on Shimadzu GC 14A gas chromatograph with flame ionization detector using 1.6 m x 3 mm (i.d.) glass column, packed with diethylene glycol succinate (15%) coated on Shimalite AW 201 (60-80 mesh). Column temperature was programmed at 150^0C for two minutes and then with a rise of 5^0C per minute to 200^0C. Injector and detector temperatures were 250^0 and 300^0C respectively. Nitrogen was used as carrier gas with a flow rate of 40 ml/minute. The fatly acids were identified by comparing their retention times with authentic methyl esters injected under the same conditions. The percentage of various acids was determined by C-R4A chromatopac computing integrator [11].

Results and Discussion

Changes of fatty acids in the samples stored for ensiling at ambient temperature 28-32^0C for 0, 30, 60 and 90 days are presented in (Tables 1, 2). Storage of the samples for 30 to 60 days showed increase or decrease in fatty acids ($C_{10:0}$ to $C_{18:3}$) while $C_{8:0}$ disappeared during this period. When the storage time was enhanced up to 90 days, fatty acid ($C_{12:0,}$ $C_{16:0}$ and $C_{18:1}$) increased more in samples No. 6, 5 and 7 respectively. This increase in the fatty acids with increase in the storage time was associated with changes in pH value from 7.0 to 4.25. The possible reason for it could be that the undissociated fatty acids were converted into dissociated fatty acids according to the following process

$$VFA \leftrightarrow VFA^- + H^+$$

The overall pH pattern in all the samples (Table 3) showed decrease after 30 days, which might be due to oxidation of sugars to carboxylic acids [12]. It increased after 60 days due to partial decomposition of fatty acids into simpler fragments and their bioconsumption by microbes present in the system. When the proton gradient increased, the reaction equilibrium shifted to the left. Since the bacterial cell wall was more permeable to un-dissociated fatty acid molecules, there was high concentration of these substances inside the cell. Once inside the cell, the undissociated fatty acids dissociate and cause decrease in pH in the cytoplasm, which in turn may inhibit enzyme activities [13]. The pH once again decreased after ensiling for 90 days (Table 3) due to dissociation of fatty acids causing increase in proton gradient and eventually decreases in pH. These results are in line with the findings of Niazi *et al.* [8] who observed similar changes in silage prepared from fruit fodder and vegetable wastes.

Table 3. pH values of silage samples prepared from different ingredients**.**

Sample No.	pH			
	0 days	30 days	60 days	90 days
1	5.0	3.8	5.6	4.3
2	4.6	3.6	5.7	4.0
3	4.7	3.7	5.6	4.2
4	4.5	3.6	5.7	4.1
5	4.8	3.8	5.7	4.3
6	4.8	3.7	5.8	4.2
7	5.4	4.2	5.3	4.5
8	5.2	4.3	5.6	4.5

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E.F., Metcalf, D., Nicola, N.A. and Gough, N.M. 1988. Myeloid leukaemia inhibitory factor (LIF) maintains the developmental potential of embryonic stem cells. *Nature* 336: 684-687

b. Chapters in books

3. **Cox, A.W.** 1988. Solar and geothermal energy. In: *Information Sources in Energy Technology.* Ed. Anthony, L.J. pp. 263-289, Butterworths, London.
4. **Smolen, J.E. and Boxer, L.A.** 1991. Functions of neutrophils. In: *Hematology.* Eds. Williams, W.J., Butler, E. and Litchman, M.A. 4th ed. pp. 3-101. McGraw Hill, New York.

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