3.5.2. Interpretation of Genomic Blot Results

Figure 2 shows a variety of genomic blot results that were obtained using the above techniques. The plants analyzed had previously been shown to be positive by PCR analysis. A complete set of analysis is shown for JKA plants 56 and 61 that had been cotransformed with construct JKA (**Fig. 2A**) and UBar (a chimeric bialaphos resistance gene driven by the maize ubiquitin 1 promoter, which was a gift of A. Christensen and P. Quail). The DNA fragment used as a hybridization probe is shown in panel A. First, the controls give the expected results; no hybridization is detected to DNA isolated from the nontransgenic, wild-type T309 (cv. Taipei 309); whereas it is detected to the four-copy positive-control reconstruction lane (4X) containing wild-type T309 DNA spiked with plasmid JKA DNA. Second, it is evident that sequences related to the probe are integrated in the genome of the transgenic plants, since all the hybridization seen comigrates with the high-mol-wt rice genomic DNA in those lanes containing undigested DNA (u).

When double-digested with *Bam*HI and *Eco*RI, plants 56 and 61 both contain the 3.2 kb fragment expected, if an unrearranged copy(ies) of the gene is present. This conclusion is confirmed by the presence of the expected 1.6 kb *Eco*RV fragments in both plants. There appear to be many more intact copies of the *Eco*RV fragment than the *Bam*HI/*Eco*RI fragment, suggesting that many rearrangement events occurred in the promoter region between the *Eco*RV and *Bam*HI sites. In addition to the expected fragments, many fragments of higher and lower mol wt are also present, indicating substantial rearrangement of the input plasmid. A rough estimate of the total number of copies of these sequences present in the rice genome can be made by estimating the number of fragments present (and their intensities compared to the reconstructions) in the lanes digested with *Kpn*I. A conservative estimate is that plant 56 has ~40–50 copies and plant 61 has well over 100 copies.

In contrast to the high numbers of copies of the gene of interest in the two JKA plants shown, the WBD plants have relatively low numbers of copies. These DNAs, including the one copy reconstruction, have been digested with *Eco*RI, which should release a 2.1-kb fragment if the 3' end of the promoter, the coding region for arcelin, and the complete nopaline synthase poly(A) addition site fragment are intact. A fragment of the correct size is present in plant 87, but not in the other two. It is unclear whether plants 68 and 76 have a functional copy(ies) of the introduced gene, with the limited data available from this single blot. For instance, if the 3' *Eco*RI site was lost during DNA rearrangement and integration, but the poly(A) addition signal was left intact, either plant could theoretically contain a functional gene. In contrast, if the *Eco*RI site in the promoter has been deleted because of rearrangement, it would